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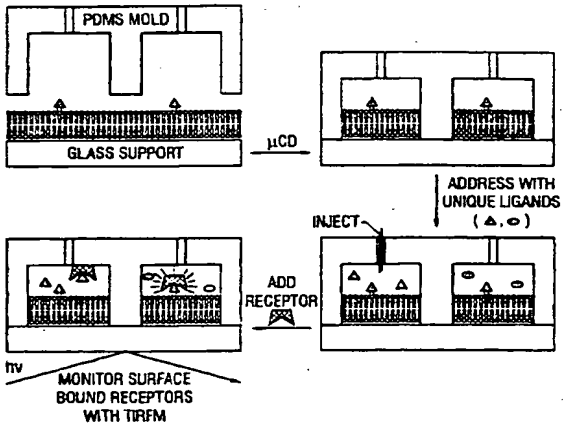

(57) Abstract: Disclosed are spatially addressed arrays of discreet fluid lipid bilayers prepared by flexible patterning methods that faciliate the compartmentalization of lipid membranes and aqueous solutions disposed thereon into discreet, spatially addressable, microarray partitions, onto specific and discreet locations of a substantially planar solid support. This process can either be used either in parallel or sequentially to pattern thousands of distinct membranes on a single "biochip", and to assay pluralities of selected analyte components contacted with the discreet lipid bilayer compartments for one or more target molecules. Also provided are biochip microarray systems and methods for their production that comprise arrays of confined aqueous compartments disposed upon such compartmentalized lipid bilayers. The aqueous compartments are independently addressable, thereby facilitating reagent delivery, reagent extraction, analytical probe and high-throughput analyte screening methods.

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SPATIALLY ADDRESSED LIPID BILAYER ARRAYS AND LIPID BILAYERS WITH ADDRESSABLE CONFINED AQUEOUS COMPARTMENTS

DESCRIPTION

1.0 BACKGROUND OF THE INVENTION

This application is a continuation-in-part of United States co-pending application serial number 09/564,708, filed May 4, 2000, which was a continuing application claiming priority to U. S. Provisional Patent Application Serial Number 60/154,576 filed September 17, 1999, and U. S. Provisional Patent Application Serial Number 60/134,210 filed May 14, 1999, the entire contents of each of which is specifically incorporated herein by reference in its entirety.

1.1 FIELD OF THE INVENTION

This invention relates to lipid microarray assay devices and systems useful in detecting and screening target molecules in aqueous solutions that interact with one or more components present in a lipid bilayer or biomembranes. The invention provides methods for creating microarray devices that comprise either (a) unique lipid bilayers disposed in spatially addressable sectors on a suitably patterned substrate, all contacted with a single analyte or target molecule, or alternatively, (b) a substantially similar single lipid bilayer disposed into discreet spatially addressable hermetically sealed microcompartments that may be contacted with a plurality of different analytes or target molecules. Using microscale design, robotic sample preparation and assay manipulations, massively parallel arrays of various biochemical assays may be performed for the compartmentalized lipid bilayers, and the resulting data quantitated, stored, and displayed *via* software- or hardware-controlled computer systems.

1.2 DESCRIPTION OF RELATED ART

1.2.1 CELL-BASED BIOSENSORS

The employment of cell-based platforms has been a very attractive strategy for developing highly selective heterogeneous binding assays and biosensors (Whelan *et. al.*, 1999; Ziegler, 2000). The use of living cells does not require one to understand the origin of the selectivity, only to properly correlate a cell's response to a particular analyte.

Unfortunately, one disadvantage of this strategy is its fragility. The cells must be kept alive in order for the sensor to function. Moreover, the expression of specific ligands may be dependent on a variety of factors including cell health, age, environment (nutrient levels, access to other cells, soluble factors, *etc.*), and cell type. Although a whole cell strategy may permit the coexistence of a great number of very different cells on a single sensor element, their instability and non-uniformity limit their use in massively parallel assay systems.

1.2.2 Fixed Ligand-Based Biosensors

By contrast, fixed ligand assays enjoy the advantage of great stability and uniformity (Sigal *et al.*, 1998; Fodor *et al.*, 1991; Brockman *et al.*, 1999; MacBeath *et al.*, 1999; Mrksich and Whitesides, 1995; Mrksich and Whitesides, 1996). Indeed, immobilized recognition elements are proving to be extremely useful for many sensor and assay designs such as DNA sequencing and enzyme recognition. However, these platforms may not be capable of the high level of selectivity needed for multivalent biological threat identification since nature does not typically employ immobilized recognition elements to detect incoming analytes. In fact native cell surfaces rely heavily on the ability of molecular recognition components to dramatically reorganize in a sea of lipids in order to maximize the number and strength of ligand-receptor interactions (Jans *et al.*, 1997; Mammem *et al.*, 1998; Kiessling and Pohl, 1996). Ligand binding on native cell surfaces can take place sequentially with chemically distinct secondary binding events occurring only after the analyte has been weakly bound to the surface. It is therefore crucial that surface ligands on mimetic sensor platforms undergo lateral diffusion in order to allow such multiple binding processes. Indeed, it has been previously shown that receptor mobility within a supported phospholipid membrane can substantially affect binding constants and signaling properties (Chan *et al.*, 1991; Tözeren *et al.*, 1992).

1.2.3 Multivalent Ligand-Receptor Binding Assays

Multivalent ligand-receptor binding is a recurring motif in the biological sciences (Kiessling and Pohl, 1996; Mammem *et al.*, 1998). Such events are ubiquitous on cell surfaces where membrane bound ligands and receptors undergo two-dimensional rearrangements to accommodate multiple interactions during a wide variety of processes. Typical examples include signal transduction and the attack of bacterial and viral pathogens

on human hosts. Therefore, the formulation of strategies that accommodate multivalent binding should be an important step in the development of a new generation of biosensors and assays that more closely mimic *in vivo* attachment at cellular membranes (Yang *et al.*, 2000; Cremer and Yang, 1999; Song and Swanson, 1999a; Song *et al.*, 1998a; Song *et al.*, 1998b; Song and Swanson, 1999b). An attractive platform for this purpose is the planar supported phospholipid bilayer (SLB) (Sackmann, 1996). SLBs, which can be formed on solid supports by vesicle fusion (Brian and McConnell, 1984) and Langmuir Blodgett methods (Tamm and McConnell, 1985), maintain two-dimensional fluidity and, hence, allow membrane-associated ligands and receptors to undergo lateral rearrangements during multivalent binding. Mobility is retained because a thin, water layer acting like a lubricant resides between the membrane and the underlying surface of this supramolecular architecture (Koenig *et al.*, 1996; Mou and Shao, 1994; Cremer and Boxer, 1999; Johnson *et al.*, 1991). When the biomimetic surface is placed in a flow cell, a heterogeneous sensor platform is created over which proteins, viruses, bacteria and other biological agents can be passed and their surface coverages monitored (Cremer and Yang, 1999; Stelzle *et al.*, 1993; Sackmann and Tanaka, 2000; Kasemo, 1998; Cornell *et al.*, 1997).

In comparison to homogeneous assays, less work has been done with heterogeneous microfluidic immunoassays. This is unfortunate as heterogeneous platforms have the added advantage of clear separation of bound and unbound species. One of the few heterogeneous examples exploits polystyrene microbeads embedded inside lithographically patterned microchannels (Sato *et al.*, 2000). Unfortunately, this assay cannot be used for *in situ* analysis nor can it undergo ligand rearrangement to accommodate multivalent receptors.

1.2.4 Lipid Bilayers as Biosensors

Lipid bilayer membranes disposed on a solid support have found recent application as components of biosensor devices (see *e.g.*, U. S. Patent No. 5,846,814). Photolithography has been used to create patterned arrays of supported lipid bilayers on a solid planar support. Because typical supported membrane devices incorporate the same membrane or phospholipid bilayer in each sector on the support, only a single assay or test may be performed using these devices, and that fact has limited their use in massively parallel fashion for screening assays involving hundreds or thousands of test solutions.

Intl. Pat. Appl. Publ. No. WO 98/23948, specifically incorporated herein by reference, discloses independently addressable supported fluid bilayer membranes. Another costly, and not wholly reproducible tissue assay currently performed involves excising the vein of a hamster, and placing it in a flow cell. These assays are tedious and cannot be performed with any significant number of compounds. Systems that include binding on SAMs (self-assembled monolayers) have been made addressable. However, in these systems, the monolayers are not fluid, a primary advantage of the invention. In addition, fluid bilayers have also been made addressable; however, each addressable sector is in contact with the identical aqueous phase. Monolayers with confined aqueous domains have been reported, but not for the study of multivalent interactions.

Consequently, there is a need for a device in which different lipid or phospholipid bilayers can be spatially addressed or otherwise compartmentalized in an organized fashion on a single substantially planar, and substantially inert support material. Devices are also needed that permit sequential, or simultaneous detection or assay of significant pluralities of target analytes present in aqueous solutions that are discreetly contacted with one or more of the compartmentalized fluid lipid bilayers using a spatially addressable lipid biomembrane array system disposed on a substantially planar support in massively parallel fashion.

2.0 SUMMARY OF THE INVENTION

The present invention overcomes the problems and disadvantages associated with prior art bioassays by providing lipid microarray assay devices having spatially addressed arrays of a plurality of substantially planar fluid lipid or phospholipid bilayers disposed on a solid support. These lipid microarray devices, and apparatus and computer-controlled systems employing them, are useful for large-scale screening and high-density throughput of massively parallel arrays of a wide variety of target analytes, including, but not limited to, pharmaceuticals, small molecules, pathogens, bacteria, fungi, microorganisms, viruses, virions, prions, peptide nucleic acids, peptides, proteins, antibodies, biomimetics, eukaryotic and prokaryotic cells, as well as natural products, chemical libraries, and/or cell components. The advantages afforded by the present invention are particularly desirable in advancing the fields of proteomics, genomics, high-throughput analyte testing, pharmaceutical drug screening, small molecule library analysis and screening, and combinatorial chemistry design and scale-up. The ability to analyze and screen biochemical interactions between target

molecules and components in a lipid bilayer in massively parallel fashion represents a substantial advance in the field of biochemistry, and for the first time, permits the analysis of multivalent interactions involving lipids and lipid membrane components on a large scale using microarray, robotic, and computer-aided control systems technologies.

In an overall and general sense, the invention provides methods for preparing lipid microarray assay devices, as well as apparatus and computer-aided systems that comprise them, that provide spatially addressed arrays of multi-component substantially planar lipid or phospholipid bilayers compartmentalized on a single substantially planar support in a massively parallel fashion.

This invention also provides methods for preparing biochips and biosensor devices having arrays of confined aqueous compartments that contact a lipid bilayer membrane disposed on a support, and, in particular, to methods for placing spatially addressable fluid lipid bilayers with confined aqueous compartments on a support, such that the aqueous compartments are independently addressable, thereby facilitating reagent delivery, reagent extraction, delivery of analytes, detectable labels, analytical probes and other such like functions employed for the biochemical analysis of one or more constituents in aqueous solution, that interact either directly, or indirectly, with one or more components of a lipid bilayer.

In a first embodiment, the invention provides a lipid microarray device that, in an overall and general sense, comprises a substantially planar, chemically inert, substrate that has on it a plurality of independently addressable isolated lipid bilayers that are discreetly positioned on the support. The support has to be sufficiently planar to allow the compartmentalized bilayers formed on the surface to be substantially isolated and independently addressable. The substrate is preferably substantially planar, and chemically inert, yet permissive to lipid adherence. The use of flat, or approximately flat, substrates facilitates the ease of manufacture and use of the compartmentalized bilayers formed thereon. Such substrates also facilitate subsequent analysis and processing steps, particularly involving replicating lipids onto the surface from a "master" plate, or replicating aquoues solutions onto the disposed lipid bilayers from a master plate, or a plurality of aqueous components or combinatorial library, or such like. Preferably, these compartmentalized lipid bilayers are positioned onto the substrate in such a fashion so that they are isolated from each other (*i.e.* the partitioned bilayer "compartments" are physically separated from each other,

and are not able to interact or commingle with any of the other neighboring bilayers partitioned elsewhere on the support). Moreover, the compartmentalized lipid bilayers are also spatially addressable, and are arrayed onto the support in such as way as to permit the introduction of aqueous solutions into the bilayer compartments without contaminating the compartments, commixing two or more lipids, or commixing the aqueous solutions disposed upon one of the bilayers with one or more adjacent compartments. Preferably, this microarray of compartmentalized lipids may be addressed by one or more means to facilitate the introduction of the aqueous solutions, to add suitable detectable labels, and/or other reagents as may be required for the performing of selected biochemical assays in the microcompartments, such as, for example, by microarray replication pins, microarray pronging devices, and/or microcapillary tubes, microfluidic delivery systems, micropipettors, syringes, *etc.* The spatial configuration of the arrayed lipid compartments preferably are such that they permit either physical manipulations by the hand of man, or alternatively, permit the intervention of one or more robotic devices or automated reagent delivery systems to facilitate introduction of the bilayers or the aqueous solutions to be contacted with the bilayers in a massively parallel array. The arrangement and number of compartments on a given device, or the size or dimensions of the physical device itself or the substrate upon which the device is prepared need not be limited to particular arrangement, number of wells, or particular grid patterns or positions, so long as each of the microcompartmentalized lipid bilayers is spatially addressable to permit the introduction and/or withdrawal of reagents, samples, labels, and the like, and that each is physically separated from any neighboring compartmentalized lipids placed elsewhere on the device. That is to say, so long as each of the compartments is isolated from each of the other compartments on the device, and that the aqueous samples to be disposed upon each of the compartmentalized lipids are not permitted to commingle or to cross contaminate neighboring wells on the array, the number and size of each of the compartments may be varied as appropriate to the particular method in which the device is to be employed. As an illustration, however, commercially popular array devices, micromanipulation devices, and microfluidic sample handling systems typically facilitate reagent delivery to arrays on the order of 96, 384, 1536, or 6144 compartments, although many other array sizes and number of microcompartments may be useful in the practice of the invention. Adaptation and scale-up may also involve direct transfer onto arrays using microfluidics and such like.

Disposed upon each of the compartmentalized independently addressable isolated lipid bilayers is at least a first aqueous solution that comprises at least a first constituent that interacts either directly or indirectly with one or more components of the lipid bilayer, and at least a second aqueous solution disposed upon at least a second of the independently addressable partitioned lipid bilayers; wherein the second aqueous solution comprises either (a) a first constituent that interacts with the lipid bilayer itself, and at least a second constituent that binds to the first constituent or to the lipid bilayer or (b) a second, distinct constituent that interacts with the lipid bilayer.

In one embodiment, the plurality of independently addressable isolated lipid bilayers may be partitioned discreetly upon the substrate by contacting the substrate with a mold that comprises a plurality of indentations, such that a hermetic seal is formed between the mold and the substrate to form the discreetly partitioned independently addressable lipid bilayer compartments upon the substrate.

The plurality of independently addressable isolated lipid bilayers disposed on the microarray device may be comprised of essentially the same biological components, or alternatively, they may be comprised of essentially distinct biological components. That is to say, all the lipid bilayers in each of the compartments of the array may be comprised of substantially the same lipid bilayer composition, or alternatively, two or more of the compartments on the array may be comprised of two or more different lipid bilayer compositions. The number of compartments on a given array device that may comprise a given lipid bilayer composition may be determined by the skilled artisan depending upon the particular assays or biochemical identification methods employed. For example, the device may be subdivided into particular areas each comprised of a given number of lipid bilayer compartments, and each of the subdivisions on a given device may contain substantially different lipid bilayer components, or each of the subdivisions on a given device may contain different concentrations of either lipid bilayer or aqueous test solution added to the compartments for assay.

The microarray device may further comprise one or more exogenous components added either to the lipid bilayer, or to one or more of the aqueous solutions disposed above the bilayers within one or more of the compartments on the device. Such exogenous component may comprise a lipid or a lipid bilayer component (such as a proteinaceous component) not normally associated with the lipid bilayer, or alternatively, the exogenous

component may comprise one or more constituents in the aqueous solutions contacted with the bilayers. Such exogenous components may include, for example, one or more detectable compounds (*e.g.*, biotinylated compounds, detectable lipid conjugates, fluorescent or epifluorescent compounds, chromogenic compounds, spin label compounds, radioactively labeled compounds, or substrates, and the like).

In one embodiment, at least two different detectable labels may be added to the lipid bilayer/aqueous solution microcompartment to facilitate the sequential and/or simultaneous detection of two or more differently labeled components. For example, the plurality of independently addressable isolated lipid bilayers may comprise one or more such detectable labels, or alternatively, the first and/or second aqueous solutions disposed upon the lipid bilayer array may comprise one or more detectable labels. In fact, in some embodiments, the target analyte or constituent to be ultimately identified or assayed, itself may be a chromogenically active compound, or may be complexed with one or more detectable ligands, or otherwise labeled in advance with one or more detectable labels. For example, when it is desirable to assay or detect the presence or absence of two or more detectable labels in a single compartment, a first detectable label may be a fluorescent label, and the second label may be a chromogenic compound. In such circumstances, the detection and quantitation of the two distinct labels may be performed either sequentially or simultaneously depending upon the detection systems present in the particular apparatus used that employs the lipid bioarray device as a primary component.

In any circumstance, substantially all of the plurality of independently addressable isolated lipid bilayers may have disposed above them a first aqueous solution that comprises the particular constituent(s) to be detected, or alternatively, a single, or only a substantially few of the plurality of bilayer compartments present on a given array device may have disposed within them a first aqueous solution that comprises the particular constituent(s) to be detected.

Likewise, in certain embodiments, substantially all of the plurality of independently addressable isolated lipid bilayers may have disposed above them a second aqueous solution that comprises a particular constituent(s) to be detected or a detectable label to facilitate the detection of the particular constituent(s) to be detected in the first aqueous solution, or alternatively, a single, or only a substantially few of the plurality of bilayer compartments

present on a given array device may have disposed within them a second aqueous solution that comprises the particular constituent(s) to be detected.

In one embodiment, it is preferable to have substantially all of the plurality of independently addressable isolated lipid bilayers contacted with an aqueous solution that comprises a second constituent to be assayed directly, or an exogenous detectable label present to permit the assay and detection of another distinct constituent in the particular aqueous solution(s) applied to the lipid bilayers in the lipid microarray device.

In another embodiment, it is preferable to have substantially all of the plurality of independently addressable isolated lipid bilayers contacted with an aqueous solution that comprises one or more exogenous component(s) such as a detectable label. Alternatively, substantially all of the plurality of independently addressable isolated lipid bilayers themselves may comprise one or more exogenous component(s) such as a detectable label.

Thus, with the devices of the present invention, it is possible (a) to label one or more components of the bilayer itself, and to detect the presence or absence of a target constituent in an aqueous solution that interacts directly or indirectly with the bilayer, by detecting the label in the bilayer, (b) to label one or more components of the aqueous solution(s) that are contacted with an unlabeled lipid bilayer, and to detect the presence or absence of a target constituent in the aqueous solution that interacts directly or indirectly with the lipid bilayer or the labeled constituent, (c) to label one more constituent(s) in the aqueous solution that interact directly or indirectly with the particular analyte being detected in the presence of the lipid bilayer, (d) to label one more of the target constituent(s) themselves, that interact directly or indirectly with one or more bilayer components, or alternatively, (e) to utilize the native physical or chemical properties of one or more target constituents themselves that can be directly detected without the need for adding an exogenous detectable label to either the lipid bilayer or to the aqueous solution in which the target analyte is present.

In a particular embodiment, when it is desirable to detect and/or quantitate the presence of a given target analyte in a plurality of aqueous solutions, the devices of the present invention typically will include plurality of independently addressable isolated lipid bilayers that are comprised of essentially the same biological components (either labeled or unlabeled), and substantially all of these compartmentalized lipid bilayers will each be contacted with a distinct aqueous solution that is suspected of containing one or more target constituents to be detected and/or assayed. The constituents in the aqueous samples to be

detected and/or assayed either (a) will themselves be capable of detection upon their interaction with the lipid bilayer (based upon inherent chemical or biochemical properties of the compounds themselves), (b) will be exogenously labeled with one or more detectable labels, that may be detected upon interaction of the target constituents with the lipid bilayer, or (c) will be in solution with one or more exogenous constituents that can be used to indirectly detect the interaction of the target constituent in solution and the lipid bilayer.

In another embodiment, when it is desirable to detect and/or quantitate the presence of a given target analyte in a plurality of aqueous solutions, the devices of the present invention typically will include plurality of independently addressable isolated lipid bilayers that are comprised of essentially the same biological components (either labeled or unlabeled), and substantially all of these compartmentalized lipid bilayers will each be contacted with a distinct aqueous solution that is suspected of containing one or more constituents that (a) bind to one or more components of the bilayer themselves, (b) bind to one or more additional components in solution that bind to the bilayer, (c) that interfere or prevent the binding of one or more components in solution to the bilayer, or (d) that promote, or increase the binding of one or more components in solution to a component of the lipid bilayer.

In embodiments where it is desirable to detect the presence of a pathogen in an aqueous sample that binds to a lipid bilayer, one may screen a library of pathogens or pathogen-derived extracts or components, such that the interaction with, or binding of, the pathogen to the lipid bilayer results in the generation of a signal that may be detected using an appropriate label (for example, by labeling a component of the bilayer or the aqueous solution, such that the binding of the pathogen to the bilayer results in a detectable change in the signal generated from the detectable label).

Exemplary lipids include, but are not limited to, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, cardiolipid, lysophospholipids, cholesterol, sphingomyelin, ceramides, gangliosides, cerebrosides (including all chain lengths and all sites of unsaturation), and the like. Furthermore, glycolipids (*e.g.* GM1), derivatized lipid conjugates (especially with ligand, fluorescent label, spin label, peptide or small molecule attachment) and polymerizable lipids are also contemplated to be useful in the practice of the present invention.

The types of proteins or polypeptides that could be present in or associated with the lipid bilayers, or alternatively, could be present in one or more of the aqueous solutions, include but are not limited to, soluble proteins covalently anchored to lipids, peripheral membrane proteins, and integral membrane proteins, derivatized proteins including myristylated, farnensylated, or any "lipidated" polypeptides, and glycoproteins. Fragments, engineered variants, and fusions of any of these proteins may also be present in the lipid bilayer or in one or more of the aqueous solutions to be assayed. Example of these types of proteins include, but are not limited to, antibodies; antibody fragments; enzymes associated with surface modification including carbohydrate processing enzymes such as glycosidases (*i.e.* sialidases, fucosidases) and glycosyltransferases (Tases) (GalTases, GlcNAc Tases, FucosylTase, SialylTase); protein processing enzymes including proteases (*i.e.* trypsin, chymotrypsin); lipid processing enzymes including lipases (*i.e.* phospholipases); enzymes responsible for transient derivatization of proteins including kinases and phosphatases; enzymes responsible for other cellular functions including DNA or RNA modification, repair, synthesis; enzymes responsible for metabolic processes including protein synthesis, secondary metabolite synthesis; proteins involved in cell regulation and signal transduction including G-coupled receptors, MHC-type, T-cell receptors (CD4/CD8)-type, dimerization domains including phosphotyrosine kinases; proteins involved in the structural integrity of a cell; proteins involved in the import of metabolites or endo/pinocytosis; toxins/toxin receptors (*i.e.* cholera toxin); ion channels (*i.e.* gramacidin). Classes of molecules that could be present include but are not limited to: single, double or triple stranded DNA/RNA; carbohydrates (monosaccharides-oligosaccharides); proteins and/or peptides; secondary metabolites; unknown samples from extracts derived from natural sources (marine, soil, organismic; *etc.*); libraries of small molecules; lipids; bioconjugates; membrane fragments of whole cells, viruses, or bacteria.

Various types of biochemical reactions can be monitored using the disclosed devices, including screening known compounds for known functions; screening known compounds for unknown functions; screening unknowns for known/unknown functions. "Function" may include but not be limited to binding, prevention of binding, enhancement of binding of additional known/unknowns, modification (*i.e.* metabolism) of the known/unknown, modification of the bilayer or components of the bilayer, affecting the aggregation state of components in the bilayer.

To scale up the process pin arrays may be combined with multi well plate formats as masters. The multi well plates are filled by either microcapillary injection, robotic injection from a picopump, injection from ink jet printer style technology, a microfluidic device, or hand delivery methods. The pin transfer consists of a variety of metal, plastic, or other style pin. Pins can be flat bottomed, slotted, pointed, and porous. They may come in uniform arrays such as, *e.g.* commercially available sizes (96, 384, 1536, 6144, *etc.*), or other sized formats. Scale-up may also involve direct transfer onto chips by any of the methods mentioned above. Microfluidics and microchannels may be used directly for scale-up.

Systems in which this technology could be employed include but are not limited to the study of cell-cell interactions (*i.e.* metathesis or inflammation or embryogenesis); cell-bacteria interactions, cell-virus interactions, cell-analyte (protein, toxin, metabolite, *etc.*) interactions; cell-polymer interactions; ligand-receptor interaction (protein-dimerization, ion channel conductance, *etc.*)

The microcompartments may also be designed with vesicles tethered on the bottom (essentially decorated on the surface) instead of supported lipid bilayers. The bilayers can also be used with polymer cushions underneath. A system using bilayers attached to colloidal gold particles, nanoparticles, or other support/sensor may also be employed. Also, any sheet of polymerizable lipids, or lipid beds containing mixtures of polymerizable lipids, lipid conjugated polymers, and free lipids could be used.

For drug screening assays it is possible to fluorescently label the molecules and use total internal reflection techniques on one of the above mentioned surfaces. On the other hand, if unknowns are brought in contact with the surface, a gold surface derivatized with thiols (hydrophilic, *e.g.* mercaptoethanol) may be utilized, the bilayer placed on top of this and then assayed by surface plasmon microscopy.

The substantially planar substrate preferably comprises a liquid-solid interface for fusing with each of the plurality of independently addressable isolated lipid bilayers.

Optionally, the substrate may further comprise a plurality of indentations capable of receiving the plurality of independently addressable isolated lipid bilayers.

The substrate preferably comprises a material selected from the group consisting of borosilicate, silica, derivatized silica, quartz, mica, thiol-functionalized gold, silicon, derivatized silicon, silicon oxide, derivatized silicon oxide, tin oxide, derivatized tin oxide, indium tin oxide, derivatized indium tin oxide, silanized silica, and $SrTiO_3$.

The plurality of independently addressable isolated lipid bilayers are partitioned discreetly upon the substrate preferably by microcontact displacement, vesicle fusion, photolithography, mechanical patterning, or etching.

When the mold comprises a substantially elastomeric material, the substrate preferably comprises a substantially rigid material. Alternatively, the mold may comprise a substantially rigid material when the substrate comprises a substantially elastomeric material.

The device of claim 2, wherein said mold comprises a material selected from the group consisting of a plastic, a polymer, a halogenated polymer, an elastomer, borosilicate, silica, derivatized silica, quartz, mica, thiol-functionalized gold, silicon, derivatized silicon, silicon oxide, derivatized silicon oxide, tin oxide, derivatized tin oxide, indium tin oxide, derivatized indium tin oxide, silanized silica, and $SrTiO_3$. Exemplary halogenated polymers include polytetrafluoroethylene (Teflon®) and polydimethylsiloxane (PDMS) represents an exemplary elastomer.

The isolated lipid bilayer may comprise one or more lipid components selected from the group consisting of a phospholipid, a cardiolipid, a lysophospholipid, a ceramide, a ganglioside, a cerebroside, a glycolipid, a sterol, a sphingolipid, and a derivatized lipid conjugate.

The isolated lipid bilayer may further comprise a non-lipid component. The non-lipid component may be selected from the group consisting of a protein, a peptide, a glycoprotein, a receptor, an antibody, an antibody fragment, an enzyme, a protease, a lipase, a phosphatase, a kinase, a nucleic acid binding protein, an ion channel, a signal transduction polypeptide, an MHC molecule, a toxin receptor, and a transport protein.

The aqueous solutions may comprise at least a first lipid, carbohydrate, polysaccharide, polypeptide, peptide, nucleic acid, small molecule component, biomimetic, antibody, antigen binding fragment, virus, bacterium, fungus, or eukaryotic cell.

The aqueous solutions may comprise a ligand, a hormone, a growth factor, a neurotransmitter, an adhesion molecule, a viral coat protein, a cell surface protein, a prion, an antigen, an extracellular matrix protein or peptide, a toxin, a cell component of a virus, bacterium, fungus, or eukaryotic cell, or a secretagogue.

The plurality of second distinct constituents may be selected from a library that is comprised of combinatorial chemistry products, secondary metabolite products, small

molecules, or natural products of animal, plant, microorganismal, viral, soil, marine, or rainforest origin.

The invention also provides an apparatus, comprising one or more of the disclosed microarray devices, and a robotic device capable of spatially addressing each of the plurality of isolated lipid bilayers partitioned discreetly upon the substrate. The robotic device may be capable of discreetly disposing the aqueous solutions upon the independently addressable partitioned lipid bilayers. The apparatus may also further comprise a program storage medium encoded with instructions that, when executed by a computer, control the robotic device. The apparatus may optionally further comprise a controller programmed with instructions for controlling the robotic device. The apparatus may also further comprise a computing system programmed with instructions for controlling the robotic device. The computing system may be part of a networked computer system.

The invention also provides an apparatus for constructing the microarray device may comprise a platform on which a substrate may be positioned; and means for discreetly partitioning a plurality of independently addressable isolated lipid bilayers upon said substrate, wherein a mold or a photolithography tool comprises, by way of example and illustration only, unique means for including a plurality of indentations on the substrate.

The invention further provides an apparatus for at least a first discreetly partitioned lipid bilayer of a microarray device, the apparatus comprising: a platform on which the microarray device may be positioned; and means for introducing the aqueous sample onto at least a first of the partitioned isolated lipid bilayers, wherein microarray of replicating pins, comprises, by way of example and illustration only, unique means for disposing an aqueous sample onto the substrate.

The invention further provides an apparatus for detecting a target constituent in an aqueous sample contacted with at least a first partitioned isolated lipid bilayer of a microarray device, the apparatus comprising: a platform on which said device may be positioned when said device contains said aqueous sample and a detectable label; and means for detecting said detectable label. In one embodiment, a detector capable of detecting a radioactive label, a spin label, a chromogenic compound, a fluorescence label, a detectable ligand, or an epifluorescent label comprises a means for detecting a target constituent in the sample.

The invention also provides a method for identifying a constituent that directly or indirectly interacts with at least a first component of a lipid bilayer, from a population of aqueous solutions. The method generally comprises applying population of aqueous solutions to the microarray device; and identifying a constituent from at least one of the aqueous solutions in the population that directly or indirectly interacts with at least a first component of a lipid bilayer disposed in the microarray device.

In alternate embodiments of the invention, a method is given for making a microarray having at least two different phospholipid bilayers. The two bilayers are separately disposed in specific sectors on the chip. This method comprises the steps of providing a hydrophilic, membrane-compatible planar substrate, preferably a planar borosilicate substrate, an indium tin oxide (ITO) substrate, a substrate of a hydrophilic thiol on gold or silver, a substrate of quartz, a silica substrate, polydimethylsiloxane or other polymer substrate, a mica substrate or other recognized membrane-compatible oxide substrates, and partitioning the substrate into a plurality of hydrophilic compartments, which are each surrounded by hydrophobic walls using a photolithographic process. The photolithographic process comprises coating a surface of the substrate with a film of photoresist, exposing the coated surface to ultraviolet light through a lithographic mask having a pattern, and developing the pattern to create the plurality of hydrophilic compartments. After partitioning, the method further comprises the steps of placing a first droplet of a first liposome solution comprising a first set of constituents (*e.g.*, lipids, cholesterol, peptides, transmembrane proteins, nucleic acids and glycosylations or phosphorylations of any of the foregoing) into a first one of said hydrophilic compartments to form a first phospholipid bilayer in said first compartment, placing a second droplet of a second liposome solution comprising a second set of constituents into a second one of said hydrophilic compartments to form a second phospholipid bilayer in said second compartment, and immersing the substrate in a buffer. Preferably, each droplet is placed in the compartments using a specially prepared hydrophobic microcapillary tube in an environment of 100% relative humidity. As will be clear to those of skill in the art, multiple wells and multiple bilayers may be disposed on a single biochip using the disclosed methods.

Another embodiment of the invention is directed to a biochip or biosensor having at least two different phospholipid bilayers comprising a substrate, a plurality of hydrophilic bilayer-compatible wells separated by hydrophobic walls disposed on the substrate, a first

phospholipid bilayer having a first set of constituents disposed in a first one of said hydrophilic bilayer-compatible wells, and a second phospholipid bilayer having a second set of constituents disposed in a second one of said hydrophilic bilayer-compatible wells.

The present invention also provides biochips and biosensors having fluid lipid bilayers with addressable, confined aqueous compartments, disposed on a single solid support. For example, one embodiment of the invention is directed to a method for making a biochip having at least two different confined aqueous compartments on a single chip. The two isolated compartments are separately disposed in specific sectors on the chip. This method for making a biochip having at least two isolated independently addressable compartments comprises the steps of providing a support, disposing a lipid bilayer substrate on the support, and partitioning the substrate into a plurality of addressable isolated compartments by disposing a mold on the support. The mold comprises a plurality of indentations, such as an array of inverted boxes or rectangular-shaped recesses. The method may further comprise the steps of placing a first droplet of a first aqueous solution into a first one of the isolated compartments, and placing a second droplet of a second aqueous solution into a second one of the isolated compartments. Preferably, each droplet is placed in the isolated compartments using a hydrophobic microcapillary tube in an environment of 100% relative humidity. The support is preferably a substantially planar support having a liquid-solid interface for fusing with the bilayer.

Another embodiment of the invention is directed to a biochip or biosensor having at least two different isolated independently addressable aqueous compartments. Preferably, the biochip comprises a support, a lipid bilayer disposed on the support, and a mold comprising a plurality of indentations disposed on the lipid bilayer. The mold separates the lipid bilayer into a plurality of isolated compartments. The chip preferably further comprises a first aqueous solution containing a first constituent disposed in a first one of the isolated compartments and a second aqueous solution containing a second constituent disposed in a second one of the isolated compartments. The first and second constituents may be different. The support is preferably a planar borosilicate. The lipid bilayer is preferably a phospholipid bilayer membrane. Preferably, the mold is a photolithographically patterned polydimethylsiloxane. The plurality of indentations in the mold may comprise an array of inverted boxes (or rectangular-shaped recesses), the boxes each having a port opposite the opening to the box to allow transfer of an aqueous solution into the isolated compartments.

The microarray devices of the present invention are most often employed in connection with robotic, automated, or data collection and manipulation apparatus. For example, one such apparatus generally comprises one of more biochips from which data are collected that are then processed by a computing system. In one aspect of the invention, the invention comprises such a computing system programmed to perform the automated and/or repetitive portions of the method. In another aspect, the invention comprises a program storage medium encoded with instructions that perform the autonomous portions of the method when executed by a computer.

The methodology disclosed herein provides for the formation of spatially addressed lipidbilayers that may comprised one or more various biological components such as lipids, phospholipids, peptides, receptors, membrane-associated proteins, glycoproteins, and the like. Although any suitably sized well plate may be used, in a preferred embodiment, square well plates from about 1 Φm or so, up to and including those sized approximately 1 or 2 cm or more, such as, for example, 25 Φm H 25 Φm to 250 Φm H 250 Φm with hydrophobic partitions ranging from 25 Φm to 250 Φm in thickness may be used, and appear to work equally well. By incorporating new deposition technologies, such as the chemical inkjet microdispenser, very large membrane libraries may be created on experimentally practical time scales (Lemmo *et al.*, 1997). In addition to being able to rapidly address a variety of membranes, the microdispenser may serve as a convenient method for depositing premixed concentration arrays of three or four component membranes analogous to the methods now being employed for materials discovery (Xiang *et al.*, 1995; Reddington *et al.*, 1998.

In another embodiment of the invention, parallel membrane deposition is used. In this embodiment, an array of microreservoirs having the same dimensions as the hydrophilic well plates is fabricated by soft lithographic techniques (Jackman *et al.*, 1998; Xia *et al.*, 1998). The reservoirs are addressed with liposome solution and employed as a master from which to transfer solution aliquots to the well plates. Droplets of solution are delivered in parallel by employing an array of metal, plastic or glass pins that can be dipped into the aligned microreservoirs and then touched to the hydrophilic well plates, in a manner analogous to pin replicator transfer of DNA arrays.

Because a substantially planar support can accommodate a wide variety of surface-specific spectroscopies, biosensors may be developed from existing technologies without the need for fluorescence labeling of lipids and proteins. One example is the application of

surface plasmon resonance imaging to detect and quantify the binding of proteins or cells to numerous surface sectors in a parallel fashion (Frutos *et al.*, 1998).

The present invention is also directed to biochips and biosensors, and to methods for making biochips or biosensors having isolated aqueous compartments above a lipid bilayer membrane. Because the compartments are individually addressable and isolated, different aqueous solutions may be disposed in each compartment, as desired.

In one embodiment of the invention, the partitioning of aqueous compartments above solid supported phospholipid bilayers is achieved by bringing a patterned polymer surface into direct contact with a supported phospholipid bilayer membrane.

For example, a phospholipid bilayer membrane may be formed over an entire planar substrate, using Langmuir-Blodgett or vesicle fusion methods. Regions of it are subsequently displaced by bringing the patterned polymer into intimate surface contact. A suitable choice of material to serve as the patterned polymer is polydimethylsiloxane (PDMS). This material is flexible enough to conform to the topography of a planar oxide or gold support and hydrophobic enough to disrupt and displace a surface supported phospholipid membrane in locations where the polymer makes intimate surface contact. The PDMS is easily molded to offer any shape desired in feature sizes ranging from 1 :m to 1 mm. Other materials (*e.g.*, polypropylene, photoresist) rather than PDMS may be used; these materials may be either hydrophobic or hydrophilic in nature to complement the surface material that supports the fluid lipid bilayer.

The present invention is not limited to glass substrates. Metallic surfaces (*e.g.*, mercaptoethanol on Au), mica, quartz, any lipid bilayer compatible substrate, fused silica, or any appropriately derivatized surface that allows the assembly of a hydrophilic bilayer would be amenable to this technique. Other appropriate surfaces include hydrophobic surfaces upon which a single bilayer leaflet is assembled.

In a preferred embodiment, the polymer surface is patterned into an array of indented boxes or rectangular-shaped recesses, and may be patterned using photolithography. Useful patterning processes include the processes described in U. S. Patent No. 5,900,160 (specifically incorporated herein by reference in its entirety). The polymer surface is then placed on the bilayer, forming a plurality of separate compartments. The mold displaces and seals the bilayer, creating a physical barrier between the compartments.

Specifically, when the polymer surface is patterned into an array of indented boxes, isolated aqueous compartments are formed with dimensions corresponding to the feature sizes present in the molded polymer. Once a PDMS mold/Bilayer/Solid Support sandwich has been fabricated, each aqueous compartment can be separately addressed by injecting solutions into it using a pulled microcapillary tube.

Since pulled glass tubes are quite brittle, the PDMS surface is preferably additionally patterned with 25 μm holes in registry with the indented boxes to facilitate injection. Massively parallel injections of different aqueous solutions can be performed using a pin array or by using an array of capillary tubes or hydrophilic tips to transfer thousands or millions of different aqueous aliquots to the various compartments. Other strategies for delivering liquids (or solids or gases) can be envisioned: for example, injection by microsyringe or delivery by microdrops. Methods and apparatus for forming microarrays using capillary dispensers are described in U. S. Patent No. 5,807,522, specifically incorporated herein by reference in its entirety.

The present invention is not limited to a single hole for each microenvironment. Multi-hole wells can be readily prepared so that multiple reagents can be delivered, microelectrodes inserted, flow cells generated, fiber optic tips incorporated, or other probes of the appropriate dimension added. In addition, two-hole flow chambers are useful for removal of debris. Materials' manipulation through microfluidics in each microenvironment can be used for other analytical purposes. These analyses include, but are not limited to, mass spectrometry, microelectrophoresis and amino acid analysis, and the like.

The present invention may be used for a number of applications. For example, it may be used in drug screening in the following manner. First, a bilayer containing a membrane bound receptor may be uniformly fused at the liquid-solid interface of a planar support using vesicle fusion or Langmuir-Blodgett techniques. The bilayer is then partitioned into separate regions by using a patterned mold containing indented boxes with holes as described previously. In the next step, aqueous phase drug molecules are transferred into the separated compartments using an array of tips. In the following step, a single virus or other contagion is transferred into all compartments at equal concentrations, where it may bind with the membrane surface, depending on the ability of the drug to inhibit this interaction in that particular compartment.

The assays of the present invention can be with different drug inhibitors, and also allow ligand-receptor binding curves to be obtained as a function of drug concentration by running many boxes in parallel with the same drug at different concentrations.

A variety of membrane-bound "receptors" can be envisioned in connection with the present invention. These include, but are not limited to, naturally occurring proteins, lipids, glycoproteins, glycolipids, synthetic molecules including small molecules, unnatural lipid conjugates, farnyslated and geraniolated proteins and glycoproteins, simple and complex carbohydrates, peptides, derivatized (glycosylated, phosphorylated) peptides, antibodies, and antibody fragments.

Attachment to the bilayer may not necessarily occur in a "biomimetic fashion." That is, ligands for His-tagged proteins could be displayed as well as known protein- or carbohydrate-binding domains. The molecule of interest may not necessarily have to be attached to the lipid directly. Similarly, other affinity fusion techniques may be taken advantage of, including, but not limited to, biotin/avidin, maltose/maltose binding protein, and N-acetylglucosamine/wheat germ agglutinin.

As will be clear to those of skill in the art, the present invention is not limited to planar substrates, although these are most easily characterized. Non-planar, curved substrates may also be similarly patterned.

3.0 BRIEF DESCRIPTION OF THE DRAWINGS

The invention may be understood by reference to the following description taken in conjunction with the accompanying drawings, in which like reference numerals identify like elements, and in which:

FIG. 1 depicts an illustrative embodiment of the present invention;

FIG. 2A, FIG. 2B, and FIG. 2C illustrate an array of supported phospholipid membranes with isolated aqueous compartments, formed and separately addressed using a conforming PDMS mold;

FIG. 3 shows a schematic depiction of a general screening assay for monitoring ligand-receptor interactions on a supported fluid lipid bilayer in the presence of a library of soluble inhibitors. Surface specific observation is achieved using total internal reflection fluorescence microscopy (TIRFM);

FIG. 4 is a schematic image of bilayer coated PDMS microchannels on a planar glass substrate. The bilayer coats both the glass and polymer surfaces. Protein solutions can then be injected into the channels as indicated by the arrows;

FIG. 5 shows a schematic layout of the TIRFM imaging setup. 1 mW of 594 nm radiation from a HeNe laser will be used to excite surface specific fluorescence. The sample is optically coupled to the dove prism by placing a drop of index matching fluid between the bottom of the float glass and the prism;

FIG. 6 illustrates one particular embodiment of an apparatus with which the invention may be practiced, the apparatus including one or more lipid microarray devices and a computing system;

FIG. 7 illustrates one particular embodiment of a networked computing system such as may be used in accordance with some alternative embodiments;

FIG. 8 illustrates one particular embodiment of an apparatus with which the invention may be practiced, the apparatus including one or more lipid microarray assay devices and a computing system, the computing system being networked; and

FIG. 9 shows the incorporation of microchannels into PDMS molds that allow the sequential flow of multiple solutions through the system. This enables supported bilayers to be formed in a first step followed by the introduction of various aqueous solutions in a second step.

While the invention is susceptible to various modifications and alternative forms, specific embodiments thereof have been shown by way of example in the drawings and are herein described in detail. It should be understood, however, that the description herein of specific embodiments is not intended to limit the invention to the particular forms disclosed, but on the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the invention as defined by the appended claims.

4.0 DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

The present invention provides novel spatially addressable fluid lipid bilayers disposed on a solid substantially planar support having confined aqueous compartments disposed there above. The present invention allows for the generation of arrays of aqueous compartments above a supported lipid bilayer membrane, and provides methods for detecting one or more selected target molecules present in those aqueous compartments. These

compartments are independently addressable with respect to reagent delivery, reagent extraction, analytical probes, and assay data acquisition and recording. The compartments can be generated in any desired shape over a range of dimensions, including, for example, from about 1 μm^2 to about 1 mm^2 or so, although the ultimate size of the individual partitions depending largely upon the number of partitions desired and the final size of each partition with respect to the size of the overall assay device itself. The compartments could range in size on the order of from about 5 μm^2 to about 0.5 mm^2, or alternatively, could have dimensions ranging from about 10 μm^2 to about 500 μm^2 or so. In fact, the size and particular dimensions of the compartmentalized lipid bilayers could range in size on the order of from about 1 or 2 μm^2, or smaller, up to and including about 5 or 10 mm^2 or even larger. The inventors contemplate that the compartmentalized lipid bilayers could range in size on the order of from about 2 or 3 μm^2, to about 4 or 5 μm^2 or alternatively, about 7, 8, 9, or 10 μm^2, and could be substantially larger on the order of about 20, about 25, about 30, about 35, about 40, bout 45, about 50, about 60, about 70, about 80, about 90, or even about 100 or so μm^2. Indeed, in certain embodiments, the compartmentalized microarrayed lipids may occupy a compartment size with dimensions ranging from about 200, 300, 400, 500, 600, 700, 800, 900, or 1000 or so μm^2 to those having dimensions of about 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or even 2000 or so μm^2. Such larger compartments may be particularly desirable when employing channel-shaped, cylindrical, or rectangular compartments for the "race track" methods disclosed herein.

When substantially larger pluralities of compartmentalized lipids are desired on a microarray, even smaller compartments may be employed, so long as they can be isolated and separately addressable within the context of the device. In those cases, the compartments could range in size on the order of from about 1 μm^2 to about 0.01 μm^2, or alternatively, could have dimensions ranging from about 0.10 μm^2 to about 0.05 μm^2 or so. In fact, the size and particular dimensions of the compartmentalized lipid bilayers could range in size on the order of from about 0.1 or 0.2 μm^2, or smaller, down to and including about 0.005 or 0.010 μm^2 or even smaller. Such devices may contain as little as two compartments, or as many as several tens, hundreds, thousands, or even millions of compartments. The plurality of compartments, while each discreet and separate from neighboring compartments, may nevertheless comprise substantially the same lipid component in each compartment, or alternatively may comprise substantially different lipid

components in two of more of the compartments. A single microarray device may contain substantially the same lipid component in each compartment, whereby the device is contacted with a library, collection, or plurality of individual discreet aqueous samples to be assayed or screened, or the device may be partitioned into two or more sections, with each section comprising a plurality of spatially addressable fluid lipid bilayers compartmentalized thereon. In such fashion, the partitioned sections may all be screened with substantially similar aqueous solutions, or each partition within a section can be contacted with a unique aqueous solution.

In any configuration of the apparatus, however, the inventors contemplate that although the individual lipid bilayer components remains fluid in each compartment, mixing of two or more adjacent compartments on the microarray device is prevented between compartments. Mixing, however, within an individual compartment of the fluid lipid bilayer component disposed therein, and the aqueous test solution contacted therewith, is freely permissible, and may be enhanced by gentle movement of the microarray device itself after the device has been loaded with lipids and target analytes, but prior to assaying the target constituents in each micro-compartmentalized assay. Such movement may be accomplished manually, or may be facilitated with one or more automatic mixing or mechanical motion inducing means, such as for example, by robotic intervention.

The arrays of the present invention provide useful technology for industry. For example, combinatorial libraries of small molecules designed to prevent surface attachment of infectious agents can be probed for activity in massively parallel arrays, allowing polyvalent attachment of ligand and receptor (biomimetic), and under a variety of experimental conditions: flow or static environment; temperature; metal ion and salt concentration; density of receptor; concentration of ligand; concentration of inhibitor; composition (fluidity) of bilayer.

In addition, arrays of the present invention are useful as tools for understanding the molecular mechanisms of disease, membrane biogenesis, and cell-cell, or receptor-ligand interactions. For example, receptor dimerization, binding of small molecules to surface receptors, can be studied under a variety of experimental conditions.

The substantially planar supported geometry of the present invention allows any number of surface specific spectroscopic events to be exploited for monitoring the binding process including, but not limited to, the following examples. In one example, on a

transparent oxide surface such as borosilicate, total internal reflection fluorescence microscopy (TIRFM) can be used to monitor surface binding. In this case the virus may be fluorescently labeled. On the other hand, surface plasmon microscopy (SPM) may be utilized to monitor binding without the need for fluorescent labeling on an appropriately functionalized gold-coated surface.

The molding technique of the present invention allows for the generation of confined aqueous domains in a variety of dimensions. In addition to the substantially cube-shaped wells described above, long channels may alternately be prepared for application in the "race track" assay. In this manifestation, strips of fluid phospholipid bilayers containing receptor sites may be formed parallel to each other in separated channels. That is, these channels may be used under flow conditions to monitor adhesion and rolling of cells along the lipid bilayer. Soluble molecules are then flowed continuously through the channel. The contagion of interest is introduced and the process is monitored using spectroscopic techniques, including optical microscopy.

An important characteristic of the present invention is that lipid bilayers may be deposited in parallel at the liquid-solid interface to form supported fluid planar bilayers in a two-dimensionally confined geometry. Confinement of a fluid membrane is accomplished by erecting barriers to lateral mobility using a polymer mold in intimate contact with the surface. In this way, biological membrane components are free to move within the confines of a single surface region, but do not cross over into neighboring regions. This strategy separates and confines the aqueous compartments above the bilayer and allows for fabrication of thousands or even millions of fluid addressable phospholipid membranes side by side on a single 1 cm^2 biochip. Previously, available patterned bilayers were chemically identical with aqueous compartments above them. These limitations prevented their use in massively parallel assays.

In addition to biological relevance, the assays of the invention are efficient and reproducible. The assays of the present invention combine the hi-throughput nature of a combinatorial assay, with the fluid nature of a real lipid bilayer. Additionally, the technology offers a simpler, safer, and more homogenous testing regime than many of the animal models currently in use (*e.g.*, the rodent vein harvesting assays employed in arthritis models). Indeed, by incorporating the appropriate cellular receptor(s), the supported bilayers of the present invention bind ligands in an environment quite similar to the one available *in*

vivo, because the membrane-based sensor and its components remain mobile on the test surface. Furthermore, the invention allows for the greatest generality. That is, the concentration of receptor, concentration of ligand, lipid composition of the membrane, pH of the environment, salts and metal ion concentrations, as well as the temperature of the experiment can be varied.

The present invention is not limited to assays of soluble or surface-bound molecules, but also provides an opportunity to exquisitely control the dimensions and application of the compartments. For example, an anti-arthritis assay system can exploit fluid flow conditions. The receptor protein E-selectin may be incorporated into the bilayer. A mold in which the aqueous compartments are designed as long channels through which reagents and buffers can be flowed is utilized. White blood cells and drug candidates may be delivered to one end of the channel and put under flow conditions by continuously pumping ligand in buffered saline. Leukocyte adhesion may be monitored with optical and fluorescence microscopy. This embodiment demonstrates a number of advantages of the present invention, including massively parallel assays for the simultaneous measurement of polyvalent surface absorption events under physiological flow conditions with a library of drug candidates.

During loading of the compartments with aqueous solutions, each droplet is preferably placed in the compartments using a hydrophobic microcapillary tube in an environment of essentially 100% relative humidity, although conditions employing about 95%, about 90%, about 85% or even lower relative humidity conditions may be desired. However, in preferred embodiments, the assays are preferably performed in an environment of essentially 100% relative humidity. As will be clear to those of skill in the art, any desired number of compartments and any desired number of bilayers may be disposed on a single support using the disclosed methods. The quantity of the aqueous solutions to be contacted with the bilayers may be on the order of 1 or 2 picoliters (pL) or even less, depending upon the sensitivity of the assay and the concentration of components, etc. In some cases, the aqueous solutions may be on the order of 3, 4, 5, 6, 7, 8, 9, or 10 pL, or so, and may be on the order of about 20, 30, 40, 50, 60, 70, 80, 90, or even 100, 200, 300, 400, 500, 600, 700, 800, 900, or even 1000 picoliters or more. In highly sensitive assays, and in assays in which large quantities of the analyte to be detected are present, substantially smaller aqueous samples may be contacted with the compartmentalized lipid bilayers. Thus, in those

embodiments, the aqueous components may be present in a volume of only 0.1, 0.01, or 0,001 pL or less.

Likewise, the volume of lipid bilayers in the compartments may vary depending upon the particular assay. In some instances the quantity of the lipid bilayer disposed with each compartment may be on the order of 1 to 5 pL, and in some cases may be on the order of 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, or even about 100 pL or so. In fact, the quantity of lipid bilayer in each compartment may be on the order of about 100, 200, 300, 400, 500, 600, 700, 800, 900, or even 1000 pL or more. In certain assays, substantially smaller aliquots of lipid bilayer may be compartmentalized onto the device. In those embodiments, the lipid components may be present in a volume of only 0.1, 0.01, or 0,001 pL or less. Likewise, in certain assays, substantially larger aliquots of lipid bilayer may be compartmentalized onto the device. In those embodiments, the lipid components may be present in a volume of 1000, 2000, 3000, 4000, 5000, 6000,7000, 8000, 9000, or even 10,000 or so pL or more.

As embodied and broadly described herein, the present invention is also directed to methods for making lipid bilayer microarrays, and for assaying arrays of multi-component planar phospholipid bilayers on a single lithographically patterned support, or alternatively, on a substantially uniform support which has been divided into compartments by the application of a mold onto the support such that the mold forms a seal with the support preventing the mingling of individual compartments within the device. The present invention is useful as a biosensor to simultaneously detect in parallel multiple targets, such as small molecules, viruses, antibodies and even cells, and cell components or extracts.

The present invention allows the user to successfully spatially address a plurality of different biomembranes on a solid support. Although previous devices have incorporated patterned arrays of supported lipid bilayers using photolithography, these devices have typically used the identical membrane in each sector. In contrast, one embodiment of the present invention allows a different membrane to be placed in each sector on the support in a massively parallel fashion, or alternatively, allows the placement of the same membrane component on the support to which a plurality of unique aqueous samples may be added. The present invention further provides a method for the flexible and general creation of addressed arrays of supported lipid bilayers.

The success of the present invention has been confirmed by epifluorescent images of multi-component supported phospholipid bilayers. In addition, the planar addressed bilayer

devices of the present invention have been placed into a standard flow cell and used successfully as biosensors.

Because the cellular membrane is the most sophisticated surface detection system ever designed, the present invention provides a facile and flexible method for spatially addressing fluid biomembrane arrays arranged on a patterned substrate in massively paralled fashion.

The present invention provides a simple and flexible method for spatially addressing fluid biomembrane arrays onto lithographically patterned surfaces with different biological components at each location. The membrane sectors in the supported lipid bilayer array may be on the micron length scale (*e.g.*, approximately 50 μm H 50 μm) allowing one to pattern ten thousand or more different fluid biomembranes onto a single "biochip" approximately 1 cm^2 in size. Such devices can be used to detect in parallel everything from small molecules to viruses, antibodies and even cells.

In one embodiment of the invention, fluid phospholipid membranes are addressed on lithographically patterned planar oxide supports by direct transfer of liposome solutions into hydrophilic bilayer-compatible wells. The hydrophilic wells may be of any desired size and shape, such as square well plates measuring about 50 μm or so in length. The hydrophilic wells are partitioned by hydrophobic spacers or barriers.

Specifically, in an illustrative embodiment, planar substrates are partitioned into arrays of micrometer sized hydrophilic boxes using standard etching techniques such as photolithography. The initial surface is substantially hydrophobic and comprises an approximately 5000 Å thick film of photoresist material spun onto an approximately 1000 Å thick chrome layer on a suitable substrate, such as borosilicate glass.

Patterning may be achieved by exposing the surface to ultraviolet light through a lithographic mask consisting of an array of 50 μm H 50 μm boxes. Developing the pattern and cleaning the substrate in an Ar^+ plasma forms well plates of hydrophilic glass onto which picoliter-sized droplets (approximately 10-100 pL) of liposome solution may be placed.

In one embodiment, the liposomes comprise small unilamellar vesicles (SUVs) of phospholipids, present at 1 mg/ml concentration in a pH 7.0, 100 mM sodium phosphate buffer solution.

Picoliter-sized solution droplets are injected onto each well plate using microcapillary tubes. The liquid quickly spreads to conform to the plate's shape while the lipid vesicles

simultaneously fuse to the underlying surface to form a continuous supported phospholipid bilayer. By incorporation of different membrane constituents into each liposome droplet, unique biological activity may be imparted to every supported membrane.

Microcapillary tubes of less than 10 μm in outer diameter, useful for injecting the liposome droplets, are preferably prepared by pulling in a standard micropipette puller (model p-97, Sutter Instruments). Prior to being filled with vesicle solution, the newly formed tubes are heated in an oven to approximately 75EC with 1,1,1,3,3,3-hexamethyldisilazane vapor. This process renders the tubes hydrophobic, which facilitates liquid transfer to the surface. The glass substrate is cooled to the dew point during the transfer process to prevent the vesicle containing droplets from losing water through evaporation; however, any method that creates an atmosphere that is substantially 100% relative humidity will work equally well. The transfer process itself may be achieved by positioning the capillary tip over the patterned oxide substrate using a linear travel stage and delivering an aliquot of liposome solution onto the surface. Droplets of different composition may be placed in each hydrophilic well plate consecutively by changing tips or simultaneously by using multiple capillary tubes separated by 50 μm and brought into registry with the patterned substrate. In another embodiment, arrays of plastic or metal pins may be used as a replicator to transfer an addressed array of vesicle solution from a master to an array of hydrophilic well plates.

After an array of vesicle droplets has been addressed on the surface, the entire system is immersed in buffer solution inside a flow cell and excess lipid vesicles are washed out. The substrate is then placed into a flow cell that allows excess liposomes to be washed away. Solutions containing different proteins may then be introduced to the substrate where binding to the fluid bilayer occurs with high specificity.

The use of planar supports for presenting large arrays of spatially addressed molecules is one of the most powerful and versatile methods for creating combinatorial libraries (Fodor *et al.*, 1991; Xiang *et al.*, 1995; Reddington *et al.*, 1998). These systems are starting to form the basis for a new generation of rapid screening assays and sensor devices in the biological and chemical sciences. In most cases, either direct covalent attachment of the desired molecules to the underlying support or simple deposition can organize the materials of interest into particular surface sectors. Extending this approach to supported phospholipid bilayer membranes containing peptides, receptors, and integral membrane

proteins is an especially valuable goal because of the ability of these systems to mimic many of the properties of native cell surfaces (Sackmann, 1996; Tampe' *et al.*, Cambridge University Press, NY, 1996).

Addressing biomembrane mimics on planar supports, however, presents unique challenges, as the two-dimensional fluidity of the biomembrane must be preserved in many cases for it to function properly (McConnell *et al.*, 1986; Watts *et al.*, 1986; Tözeren *et al.*, 1992). This requires the bilayer deposition process to take place in an aqueous environment and that the entire system continues to remain submerged underwater to preserve the planar supported structure. Because of this physical constraint as well as the inherent complexities of biomembrane materials, traditional technologies, such as light-directed synthesis for addressing peptide or DNA sequences onto solid supports, are inherently more difficult to apply (Fodor *et al., Science,* 251:767, 1991). The present invention, therefore, employs an alternate approach based upon depositing mesoscopic quantities of aqueous solution onto lithographically patterned hydrophilic surface well plates (Kumar *et al.*, 1994), followed by the immersion of the entire substrate into buffer. This provides a general and flexible method for directing chemically distinct phospholipid membranes into individually addressable surface sectors.

Phospholipid vesicles spontaneously assemble into single supported planar bilayers of approximately 5 nm in height at the solid-liquid interface (Sackmann, 1996; Tampe' *et al.*, Cambridge University Press, NY, 1996; McConnell *et al.*, 1986; Kalb *et al.*, 1992); however, the choice of solid support profoundly affects the process (Rädler *et al.*, 1995; Keller *et al.*, 1998; Cremer *et al.*, 1999). Silica, borosilicate, and PDMS based substrates are well suited for this purpose since the bilayer material adheres to the substrate by weak van der Waals interactions and allows a thin water layer (1-2 nm) to remain between the membrane and the substrate (Bayerl *et al.*, 1990; Johnson *et al.*, 1991; Koenig *et al.*, 1996. These surfaces can be patterned with membrane incompatible materials or by scratching the surface to laterally partition one lipid bilayer from the next (Groves *et al.*, 1995; Groves *et al.*, 1997; Groves *et al.*, 1998). Molecules within an individual membrane are free to move within the confines of a partition, but do not cross over to a neighboring region. The present invention exploits this idea in combination with microlithography to allow large arrays of fluid bilayer membranes to be spatially addressed in neighboring corrals on a single surface.

4.1 MICROCONTACT PRINTING

Whitesides and coworkers have described microcontact printing (μCP) as a strategy for patterning arrays of self assembled monolayers by employing a polydimethylsiloxane (PDMS) elastomer coated with alkanethiol and stamping the materials onto a gold substrate (Kumar and Whitesides, 1993). Biebuyck and coworkers extended the use of elastomers to generate microfluidic networks by bringing lithographically patterned PDMS molds, which had been rendered hydrophilic by oxidation, into contact with solid substrates such as Au, glass, or silica/Si (Delamarche *et al.*, 1997). PDMS formed the walls and roof of the sealed microcompartments while the floor consisted of the substrate. By derivatizing the substrate surface and flowing in the appropriate biological or chemical components, elaborate thin films of varying composition could be formed on the surface with micron level resolution. Microarray printing devices have also been described in U. S. Patent No. 6,101,946, miniaturized cell array and robotic systems have been described in U. S. Patent No. 6,103,479, and the use of microarrays in the multiplexed analysis of biomolecules has also been described in U. S. Patent No. 6,083,763 (each of which is specifically incorporated herein by reference in its entirety).

4.2 COMBINATIONAL LIBRARIES

There is great impetus for creating combinatorial libraries of solid supported fluid lipid membranes because these systems retain many of the properties of native cell surfaces (Tözeren *et al.*, 1992; Chan *et al.*, 1991; Sackmann, 1996). Presenting ligand arrays on fluid bilayers can be exploited for the development of biosensors, screening assays, as well as for use in understanding fundamental processes of ligand-receptor interactions. Recently we have shown that supported arrays of fluid phospholipid membranes can be formed on planar solid supports with unique chemical constituents at each address by using a combination of photolithographic patterning and microcapillary injection (Cremer and Yang, 1999). This technique creates a patterned array of chemically distinct bilayers, each of which is in contact with the identical aqueous solution.

An even more desirable process addresses aqueous solutions above an array of planar supported bilayers. In combination with surface specific detection, this strategy enables the rapid screening of a library of soluble molecules for their efficacy in inhibiting ligand-receptor interactions in a fluid membrane environment that is similar to *in vivo* conditions.

4.3 Implementing the Invention in Software on a Computer System

As may be apparent from the present disclosure, when preparing biochips with large numbers of addressable compartments, the manipulation and storage of data obtained from each of the compartments can become cumbersome if done manually. This is particularly true for large arrays of samples, which are used in the screening of large numbers of aqueous samples. In such cases, the inventors contemplate the use of the disclosed microarray devices in automated apparatus and systems designed to facilitate obtaining, manipulating, and storing the plurality of data obtained in these methods. As such, robotic sampling equipment, computer systems, and data storage media may all be employed to utilize the disclosed bioassay devices in a large-scale, or high-throughput embodiment. Typically, in such large-scale microarray systems, some portions of the method of the invention will typically be software implemented. Illustrative embodiments of a software implementation of the invention from a functional perspective are described below. In the interest of clarity, not all features of the actual implementation are described. It will of course be appreciated that in the development of any such actual embodiment, numerous implementation-specific decisions must be made to achieve the programmers' specific goals, such as compliance with system-related and business-related constraints, which will vary from one implementation to another. Moreover, it will be appreciated that such a development effort, even if complex and time-consuming, would be a routine undertaking for those of ordinary skill in the art having the benefit of this disclosure.

Since the invention will typically be implemented, at least in part, in software, some portions of the detailed descriptions herein are consequently presented in terms of a software-implemented process involving symbolic representations of operations on data bits within a computer memory. These descriptions and representations are the means used by those in the art to most effectively convey the substance of their work to others skilled in the art. The process and operation require physical manipulations of physical quantities. Usually, though not necessarily, these quantities take the form of electrical, magnetic, or optical signals capable of being stored, transferred, combined, compared, and otherwise manipulated. It has proven convenient at times, principally for reasons of common usage, to refer to these signals as bits, values, elements, symbols, characters, terms, numbers, or the like.

It should be borne in mind, however, that all of these and similar terms are to be associated with the appropriate physical quantities and are merely convenient labels applied to these quantifies. Unless specifically stated or otherwise as may be apparent, throughout the present disclosure, these descriptions refer to the action and processes of an electronic device, that manipulates and transforms data represented as physical (electronic, magnetic, or optical) quantities within some electronic device's storage into other data similarly represented as physical quantities within the storage, or in transmission or display devices. Exemplary of the terms denoting such a description are, without limitation, the terms "processing," "computing," "calculating," "determining," "displaying," and the like.

4.3.1 AN EXEMPLARY COMPUTER SYSTEM EMPLOYING A STAND-ALONE COMPUTING DEVICE

FIG. 6 illustrates an exemplary computer system **500** including a computer **505** programmed implement the present invention in conjunction with the biosensor device **508**. Note that, in the particular implementation of the computer system **500** illustrated, the computer **505** is a desktop personal computer. However, the invention is not so limited. The computer **505** may also be, for instance, a workstation, a laptop computer, a handheld computer, or even a network server, in various alternative implementations.

Returning to **FIG. 6**, the computer system **500** includes standard input/output devices such as a keyboard **510**, mouse **520**, and monitor **530** with which a programmer may interact with the software loaded on the computer **505**. This interaction is performed in accordance with conventional practices by which programmers interface with software. The computer system **500** may include a network connection **540** so that a programmer may interface with other computers over a network (not shown) through the computer system **500**. However, this is not necessary to the practice of the invention. In the particular embodiment illustrated, the computer system **500** is interfaced with one or more biochip devices **508** over the connection **540**. Note also that the particular operating system implemented on the computer **500** is not material to the practice of the invention. The computer **500** may, without limitation, implement a Windows®, UNIX, or a Microsoft Disk Operating System ("MSDOS")-based operating system, for instance.

FIG. 6 also illustrates a magnetic floppy disk **550** and an optical disk **560**. The software of the present invention may be encoded on a variety of program storage media,

whether optical, magnetic, or otherwise in nature, such as the floppy disk **550** and the optical disk **560**. The software may also be encoded on the hard disk (not shown) of the computer **505**. Thus, in alternative embodiments, the invention may comprise instructions that, when executed by a computer, perform a method in accordance with the invention. Similarly, the invention may comprise a computer, *e.g.*, the computer **505**, programmed to implement the method.

The software of the invention can be conceptualized as an applications program **570** and one or more data structures **580**. One or both of the applications program and the data structure(s) may be encoded on one or more of the program storage media in the computer system **500**. Note that the language in which the applications program **570** is written and the type of structure in which data is stored are not material to the practice of the invention. These types of details will be implementation specific. For further information, a general treatment of programming languages and data structures, including relative strengths and weaknesses, may be found in *The Computer Science and Engineering Handbook*, pp. 86-110, 977-1190, 1981-2256 (Allen B. Tucker, Jr., Ed.-in-Chief, CRC Press, 1997; ISBN 0-8493-2909-4). Thus, the data structures **580** may be any suitable data structure known to the art, *e.g.*, a database, a linked list, *etc.*

The data structure(s) **580** may contain selected numerical results of the data output from one or more of the individual assays performed in one or more of the plurality of compartments of the microarray device. More technically, the data structure(s) **580** contain data representing numerical results of one or more of the individual assays. However, the term "numerical results" as used herein shall encompass not only the display of such data, but the data itself. The term "image" as used herein therefore does not necessarily imply that the data is being displayed. In one particular embodiment, several data structures **580** are employed. The data structure **580** in this particular embodiment contains numerical results of one or more biochemical assays performed using the compartmentalized lipid systems disclosed herein, but may, in alternative embodiments, contain digitized images or fluorescent images of one or more compartmentalized assays. Likewise, the structure may contain numerical or digital images of one or more "reference" points, such as, for example, positive and/or negative control data points, or such like. Such reference points may be used to compare the results obtained in one or more compartments to one or more standards or control data points.

4.3.2 Exemplary, Networked Computer Systems

FIG. 7 and **FIG. 8** illustrate alternative embodiments for the computer system **500** of **FIG. 6**. The invention admits wide variation in equipment, communications protocols, certain applications software, and transmission media, as will become apparent from the following discussion. Indeed, the invention is not limited by these factors. Each of these factors will be implementation specific. The particular embodiment of the computer system **600** shown in **FIG. 7** and computer system **700** in **FIG. 8**, like the computer system **500** in **FIG. 6**, are exemplary only, and are set forth to further an understanding of the invention.

The computer systems **600** and **700** are both networked. The computer system **600** comprises a plurality of networked computers **610** communicating with a network server **620** over a plurality of corresponding communications links **620**. The illustration in **FIG. 7** vaguely resembles a local area network ("LAN") configured in a star topology although the invention is not so limited. For instance, the computer system **600** may, in some embodiments, be a wide area network ("WAN") or may even comprise a part of the Internet. Indeed, not all embodiments are even networked, as is the case for the computer system **500** in **FIG. 6**. Similarly, although the particular embodiment illustrated in **FIG. 7** employs a client/server architecture, any suitable architecture known to the art may be used, *e.g.*, a peer-to-peer architecture. The computers **710** of the computer system **700** in **FIG. 8**, for instance, are configured in a peer-to-peer architecture. Note also that, in client/server architectures, more than one server **620** might be employed.

The communications protocols and types of computing devices also will be implementation specific. The computer systems **600**, **700** may also utilize any suitable communications protocol, *e.g.*, an Ethernet or a token-ring network protocol. The communications links **630**, **720** may be implemented using any suitable transmission media known to the art, including without limitation optical fibers, co-axial cables, and twisted wire pairs. In some embodiments, the communications links **630**, **720** might even be wireless links. The computers **610**, **710** illustrated are all desktop personal computers. However, the invention is not so limited. The computers **610**, **710** might be some other type of computing device, for instance, a workstation, a laptop computer, or a handheld computer.

Note that the computing systems **600**, **700** both include multiple data structures **580**. These may provide data redundancy in some embodiments. In other embodiments, however,

the data structures **580** may contain different data. More particularly, the biosensor microarray methods disclosed herein may be used to create a first data structure **580** whose data represents a first set of results and then a second data structure **580** whose data represents a second set of results obtained from a similar, or from a distinctly different assay or device. This may be extended in principle to include numerous data structures whose data manifests numerous results from a single biochip, and/or results from pluralities of two or more biochip devices, or in some embodiments, data structures whose data manifests numerous repetitions of assays on a single, or on a plurality of biochips.

4.3.3 THE SOFTWARE IMPLEMENTATION OF THE METHOD OF THE INVENTION

In accordance with standard networking principles, the situs of the applications program **570** and the data structure(s) **580** is flexible. In **FIG. 7**, the applications program **570** resides on the server **620** while the data structures **580** are distributed across the computers **610**. However, the application program **570** and data structures **580** could all reside on the same computing device **610, 620** or be distributed across the networked computing system **600** in some other manner. Similarly, the applications program **570** and the data structures **580** are illustrated evenly distributed across the network **710**, but may be distributed in some other manner. In the stand-alone embodiment of **FIG. 6**, this is not a consideration.

4.3.4 IMPLEMENTATION ON A COMPUTER SYSTEM

Thus, the particular hardware in the various embodiments of the present invention will be specific to a particular implementation. The computer system **600** may be as simple as the computer system **500** in **FIG. 6**, as intensive as a mainframe computer hosting dozens of time sharing users, or as distributed as a bunch of networked computers, *e.g.*, the computer systems **600, 700** of **FIG. 7** and **FIG. 8**. Also, although not shown, the elements of the computing system might alternatively be embedded in the echocardiograph machine. Thus, the invention is, in one aspect, a software implemented method for analyzing data as is discussed more fully elsewhere as opposed to the hardware. In other aspects, the invention comprises a program storage medium encoded with the software capable of implementing the method and/or a computer programmed to implement the method. The program storage medium may be magnetic, such as the floppy disk **560**, or optical, such as the compact disk,

read only memory ("CDROM") 570, both shown in FIG. 6, or some other type of medium capable of storing the computer instructions.

5.0 EXAMPLES

The following examples are included to demonstrate preferred embodiments of the invention. They also illustrate more fully the system and software that drives one particular embodiment of the invention disclosed above. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention described in the appended claims.

5.1 EXAMPLE 1– PARTITIONING OF SUBSTRATE AND APPLICATION OF LIPOSOME SOLUTION

Planar borosilicate substrates were partitioned into arrays of micrometer sized hydrophilic boxes using standard photolithography. The initial surface was substantially hydrophobic and consisted of a 5000 Å film of photoresist spun onto a 1000 Å chrome layer on borosilicate glass. Patterning was achieved by exposing the surface to ultraviolet light through a lithographic mask consisting of an array of square boxes. Developing the pattern and cleaning the substrate formed well plates of hydrophilic glass onto which picoliter-sized droplets of liposome solution were placed. The liposomes, which were small unilamellar vesicles (SUVs) of phospholipids, were present at 1 mg/ml concentration in a pH 7.0, 100 mM sodium phosphate buffer solution.

Bright field images of a microcapillary tube containing liposome solution situated over the array of patterned hydrophilic glass plates (50 μm H 50 μm) were obtained. The tip of the capillary tube, which is directly over the center of the lower right-hand plate, is less than 5 Φm above the surface. Microcapillary tubes of less than 10 μm in outer diameter were prepared by pulling in a standard micropipette puller (model p-97, Sutter Instruments). Prior to being filled with vesicle solution, the newly formed tubes were heated in an oven to 75EC under a vapor of 1,1,1,3,3,3-hexamethyldisilazane. This process rendered the tubes hydrophobic, which facilitated liquid transfer to the surface. The glass substrate was cooled to the dew point (8-12EC) during the transfer process to prevent the vesicle containing droplets from losing water through evaporation. The transfer process itself was achieved by

positioning the capillary tip over the patterned oxide substrate using a linear travel stage and delivering an aliquot of liposome solution (typically 10-100 pL) onto the surface. Droplets of different composition may be placed in each hydrophilic well plate consecutively by changing tips or simultaneously by using multiple capillary tubes separated by 50 μm and brought into registry with the patterned substrate. After an array of vesicle droplets had been addressed on the surface, the entire system was immersed in buffer solution inside a flow cell and excess lipid vesicles were washed out.

5.2 EXAMPLE 2 – EVALUATION OF BIOCHIPS USING EPIFLUORESCENCE

The epifluorescence image of nine 50 μm H 50 μm glass well plates containing addressed fluid phospholipid bilayer membranes that were patterned with three chemically distinct types of supported phospholipid bilayers was obtained. Each membrane was predominantly comprised of egg phosphatidylcholine (egg PC) lipids. However, the four corner boxes, appeared red in color, contained 1 mol% phosphatidylethanolamine (PE) derivatized with a Texas Red fluorophore at the head group (1 mol% Texas Red DHPE fluorescent probes). The other four outer boxes appeared green in color and contained 3 mol% of a green fluorescent lipid probe, NBD-PE (3 mol% nitrobenzoxadiazole glycerophosphoethanolamine (NBD)-DHPE probes. The center box, which appeared dark yellow, contained a 50/50 mixture of the first two types of membranes, or both kinds of fluorophores. The image was obtained with an E800 fluorescence microscope from Nikon equipped with a black-and-white charge-couple device (CCD) camera. The image was processed by the technique of false color imaging.

Fluorescence recovery after photobleaching (FRAP) demonstrated that the lipids were free to move throughout each two-dimensional box, but were otherwise completely confined (Axelrod *et al.*, 1976; Soumpasis, 1983). The bilayer in the center box was formed from a premixed solution of the NBD and Texas Red labeled lipids. When the two solutions were added to the same box sequentially, the bilayer consisted primarily of the lipids that were introduced first. This occurs because the self-assembly process is completed within a few seconds under the conditions used for these studies. Lipids in the outer leaflet of the supported bilayer will slowly exchange with those from vesicles in solution, but this process usually takes from tens of minutes to several hours at 10EC. Lipids in the inner bilayer leaflet were even less accessible (Reinl *et al.*, 1994; Brown, 1992).

5.3 EXAMPLE 3 – INCORPORATION OF BIOTIN-STREPTAVIDIN SYSTEM

Selective incorporation of a protein receptor site into the patterned membranes demonstrated the viability of patterned supported lipid bilayers as biological sensor devices. In this example, the biotin-streptavidin system was utilized, as this system has been well characterized with planar supported membranes (Calvert *et al.*, 1997). Biotin is a small molecule that can be covalently attached to the headgroup of phosphatidylethanolamine lipids and binds the aqueous protein, streptavidin, with a high affinity.

The epifluorescence images of four 50 μm by 50 μm boxes or hydrophilic well plates were obtained. The membrane in box 1 contained a fluorescently labeled membrane with 2-mol% biotinylated PE. It was formed from egg PC vesicles containing 3 mol% NBD-PE and 2-mol% biotinylated PE. The other three boxes represented control studies. Box 2 contained a fluorescently labeled membrane without biotinylated PE. It was formed from an egg PC membrane with 3 mol% NBD-PE and no biotinylated PE. Box 3 contained no membrane and was left empty. Box 4 contained a fluorescently labeled membrane with 1 mol% biotinylated PE. In other words, box 4 was similar to box 1, but the biotinylated PE concentration was only 1 mol%. The fluorescence image was intentionally taken with the room lights on so that a small amount of ambient light would leak into box 3 and outline its position.

Upon exposure of the substrate to a 10 μM concentration of Texas Red labeled streptavidin solution, the membrane patches containing biotinylated lipids quantitatively absorbed the streptavidin, which remained mobile on the surface. Quantitative analysis of the fluorescence revealed that box 1 contained twice as much protein as box 4. In contrast, in box 2, no streptavidin was detected (The signal to noise in these studies permitted visualization of concentrations well under 0.1 monolayers of fluorescently labeled protein). As expected, a small quantity of protein absorbed to the bare glass surface of box 3, but was immobile as determined by FRAP. The results not only demonstrated the high level of binding specificity of streptavidin to fluid supported membrane patches containing the biotin receptor site, but also indicated a high level of resistance of fluid phospholipid membranes to non-specific protein binding.

5.4 EXAMPLE 4 – SPATIALLY ADDRESSABLE AQUEOUS MICROCOMPARTMENTS ABOVE SOLID SUPPORTED LIPID BILAYERS USING LITHOGRAPHICALLY PATTERNED PDMS MOLDS

A soft lithographic technique (Xia and Whitesides, 1998) has been employed for partitioning and addressing aqueous solutions above supported lipid bilayers to form microarray devices that may be employed in multivalent screening assays to analyze pluralities of analytes. This highly flexible methodology affords the ability to create a large number of aqueous compartments consisting of various chemistries, pH values and ionic strengths, as well as ligand and inhibitor concentrations above patterned arrays of lipid membranes (Groves *et al.*, 1997; Kumar and Whitesides, 1993) on a single planar support.

This example demonstrates that bringing lithographically patterned PDMS molds into contact with planar supported lipid bilayers in aqueous solution leads to highly selective microcontact displacement (μCD) of the phospholipid membrane from the substrate. Leaving the mold in place creates sealed and addressable aqueous compartments above patterned membrane arrays.

To functionalize the substrate, a continuous fluid egg PC bilayer containing 1 mol% Texas Red DHPE was formed on a planar borosilicate support using the vesicle fusion method (Brian and McConnell, 1984). A lithographically patterned PDMS mold containing a grid pattern of 60 μm thick raised lines was gently placed in contact with the supported bilayer and the system was observed underneath an epifluorescence microscope. As can be seen strikingly in the fluorescence images, the bilayer was displaced from the solid support where the mold makes conformal contact with the surface. For the images shown, the process takes place over a period of about 90 min., although it could be made to occur in less than 5 min. by applying additional pressure. Quantitative fluorescence measurements based on line profile data indicated that multiple bilayers were not formed during the process. Instead, the majority of the membrane material displaced from the surface formed free vesicles in the aqueous solution. Two additional fates of bilayer material also have been observed. When the mold is not substantially flat enough to make good conformal contact with the entire surface of the substrate, some material becomes trapped between the mold and the substrate surface. Furthermore, in some cases, if the mold is not substantially contacted with the entire surface of the substrate, a small amount of lipid material sometimes was observed to congregate where the bilayer sectors meet the elastomeric wall of the mold itself. This latter material can be easily rinsed away upon removal of the mold.

Interestingly, μCD occurs in the presence of both hydrophobic and hydrophilic (freshly oxygen plasma treated) (Delamarche *et al.*, 1997) PMDS molds. Apparently, the interaction between the mold material and the underlying glass surface is sufficiently strong to force bilayer displacement under a variety of surface chemistries depending upon the type of mold used. The partitioned bilayers remain fluid on the surface with a diffusion constant of approximately 4×10^{-8} cm^2/sec as determined from the FRAP technique (Axelrod *et al.*, 1976; Soumpasis, 1983).

An important consequence of the partitioning procedure is the formation of individually isolated aqueous compartments disposed above each of the compartmentalized lipid bilayers on the substrate. These aqueous compartments can be separately addressed by patterning small holes in the portion of the elastomer that serves as the roof above the confined aqueous compartments. For this purpose circular holes were made which were 100 μm in diameter (Duffy *et al.*, 1998). Pulled microcapillary tips mounted on an XYZ translation stage are used to transfer solutions into the holes under the microscope (Cremer and Yang, 1999). Epifluorescence images of supported lipid bilayer patches containing 1 mol% Texas Red labeled lipids that were patterned by contact with a PDMS mold, and injection of fluorescein dye into the upper right hand box clearly demonstrated that the aqueous compartment above this bilayer was sealed from it neighbors.

To demonstrate the utility of this partitioning procedure for screening libraries of small soluble molecules for their ability to inhibit binding between surface bound ligands and aqueous phase receptors, a supported phospholipid bilayer was formed containing 2 mol% biotinylated lipids and placed in contact with the PDMS-patterned surface. Biotin is a small molecule that binds the soluble protein, streptavidin, with high affinity and has been well characterized with planar supported lipid bilayer systems (Calvert and Leckband, 1997). Eight nanoliter droplets of 1 mM N-2,4-DNP-glycine, 1 mM bovine serum albumin, and 1 mM biotin PEO-amine were added to the aqueous compartments in boxes 1, 2, and 3 respectively (the final concentrations of these species are attenuated by approximately a factor of 20 through dilution as the aqueous container volumes were roughly 150 nL). Compartment 4 was left unaltered as a control. Upon addition of 8 nL droplets of a 10 μM concentration of fluorescently labeled streptavidin to all four of the compartments, it could be seen that the fluorescence from the aqueous phase (some streptavidin was observed to adsorb to the walls of the PDMS mold; this process could be suppressed by pre-adsorbing

bovine serum albumin as suggested in Delamarch *et al.* (1997)) above each of the bilayers looked identical. Using the evanescence wave generated from the 594 nm line of a HeNe laser to excite only the surface bound, Texas Red labeled streptavidin (Alexrod *et al*, 1984) reveals that a high concentration of streptavidin is bound in boxes 1, 2, and 4 while the surface of box 3 remains protein free. These results demonstrated the ability to deliver soluble analytes to these arrays multiple times, and to evaluate their ability to prevent surface absorption events. This methodology is generally applicable to investigating the efficacy of candidate drugs to inhibit multivalent binding of ligands and receptors[18-19] in a fluid environment.

5.5 EXAMPLE 5 –SUPPORTED PHOSPHOLIPID BILAYERS AND THE IMPORTANCE OF FLUIDITY

A simple example of multiple binding site interactions that occurs at cell surfaces is that between an IgG antibody and membrane associated haptens. Other examples include the binding of influenza virus to red blood cells and the binding of human immunodeficiency virus (HIV) to helper *T* cells (Mammem *et al.*, 1998).

Many of these interactions can be modeled on planar surfaces using solid supported phospholipid membranes.

Solid supported bilayers are attractive platforms for biosensor design because they allow measurements to be made in a well-controlled environment where the surface binding sites remain laterally fluid (Sackmann, 1996; Johnson *et al.*, 1991; Williams *et al.*, 1997; Stelzle *et al.*, 1992; Cheng *et al.*, 1998; Tamm and McConnell, 1985; Kalb *et al.*, 1992; Cremer and Boxer, 1999). This is possible because a thin aqueous layer approximately 1-2 nm thick is trapped between the bilayer and the underlying oxide support. This water layer acts as a lubricant allowing both leaflets of the bilayer to remain fluid (Johnson *et al.*, 1991). Consequently, planar supported membranes retain many of the properties of free vesicles or even native cell surfaces when the appropriate recognition components are present. They are convenient to study with a host of surface-sensitive techniques (Sackmann, 1996) and are far less fragile than cell-based assays. Supported bilayer membranes can be formed by either Langmuir Blodgett methods or through the fusion of small unilamellar vesicles to the planar solid substrate (Tamm and McConnell, 1985; Kalb *et al.*, 1992). Either way it is relatively straightforward to present appropriate ligands and receptors at these model membrane

surfaces. Any number of species including peptides, peptide labeled lipids, biotinylated lipids, channel forming proteins, and antigenic ligands have been presented in supported bilayers (Sackmann, 1996).

5.6 EXAMPLE 6 – MICROARRAYS AND MICROFLUIDIC DEVICES

Fluid bilayer-based platforms for sensor design will be even more powerful once methods have been developed for facilely incorporating them into spatially addressed microarrays (Fodor *et al.*, 1991; Brockman *et al.*, 1999; MacBeath *et al.*, 1999; Cremer and Yang, 1999; Martin *et al.*, 1998). Microarray technology has inspired the design of complex massively parallel biosensors ever since Fodor and coworkers first demonstrated that light directed synthesis could be employed to address immobilized biomaterials on planar surfaces (Fodor *et al.*, 1991). This technology makes it possible to rapidly screen large libraries of compounds for specific chemical or biological activity in a single experiment. These systems are seeing particularly heavy use in the biotechnology arena, where libraries of surface addressed DNA strands are being employed to rapidly screen free DNA fragments.

Microfluidics is another growing technology (Jacobson *et al.*, 1999; Hosokawa *et al.*, 1999; Duffy *et al.*, 1998; Xia and Whitesides, 1993; Kenis *et al.*, 1999; Colyer *et al.*, 1997; Chiem and Harrison, 1998; Duffy *et al.*, 1999; Delamarche *et al.*, 1997; Schasfoort *et al.*, 1999; Yang *et al.*, 2000). It involves the flow and manipulation of liquids in micron-sized channels for rapid chemical and biochemical analysis. Such channels can be combined with micro-valves and actuators that are lithographically printed or mechanically micromachined into the device. Developing integrated microfluidic systems makes it possible to direct, mix, and monitor picoliter quantities of biofluids within a single chip platform. This is of great interest because small integrated units for sample processing (often referred to as laboratories-on-a-chip) hold out significant promise for use in genomics, drug discovery, biochemical sensing, and other related fields where rapid, parallel analysis of tiny quantities of analytes is desirable (Colyer *et al.*, 1997; Chiem and Harrison, 1998; Duffy *et al.*, 1999; Delamarche *et al.*, 1997; Schasfoort *et al.*, 1999; Yang *et al.*, 2000). Microfluidic devices have several additional intriguing properties. For example, the flow of liquids through these systems is normally laminar with Reynolds numbers typically well less than 100 (Kenis *et al.*, 1999). Additionally, the surface area to volume ratio is quite high compared to macroscopic systems, causing a much larger fraction of an aqueous analyte to be in contact

with the device's surface at any given time. Finally, the small size scale itself makes it possible to perform hundreds and perhaps thousands of tests on a single chip simultaneously.

The substrates in microarrays and microfluidic devices are typically made of polymethyl methacrylate (PMMA), polydimethylsiloxane (PDMS), float glass, or similar materials. In the case of microfluidic devices, these surfaces may be used bare if all the processes of interest take place in solution, although the experiments may suffer from nonspecific surface adsorption (Delamarche *et al.*, 1997). If the surface is to take an active part in the process, these architectures need to be derivatized with chemical or biochemical recognition elements, which can be covalently bonded to the device's walls and floor. For example, tricholorosilanes work well to attach functional groups to glass surfaces through hydrocarbon or ethylene oxide tethers (Ulman, 1996; Wasserman *et al.*, 1989). More sophisticated immobilization chemistries are also in common use (Atheron and Sheppard, 1989). Of course, if the substrate is gold, then thiol based self-assembled monolayers can be formed (Bain *et al.*, 1989).

5.7 Example 7 – Spatially Addressed Phospholipid Bilayers Arrays

Combining solid supported membranes with the techniques of microlithography has allowed these systems to be partitioned into arrays of isolated membrane patches (Groves *et al.*, 1997; Groves *et al.*, 1998). Individual membrane components are free to move throughout a particular sector, but are otherwise confined. This example describes a novel strategy for membrane addressing based upon depositing mesoscopic quantities of vesicle solution onto lithographically patterned hydrophilic surface well plates, followed by the immersion of the entire substrate into buffer. The methodology is both general and flexible so any combination of phospholipids, cholesterol, peptides, carbohydrate labeled lipids and proteins, or other derivatized membrane components can be placed at any desired location.

Fluid phospholipid membranes are addressed on lithographically patterned planar oxide supports by direct transfer of liposome solutions into the hydrophilic well plates. These square-shaped plates are usually 25 to 250 μm on a side and partitioned by hydrophobic spacers. Picoliter sized solution droplets can be directly injected onto each well plate and the liquid quickly spreads to conform to the plate's shape while the lipid vesicles simultaneously fuse to the underlying surface to form a continuous supported phospholipid bilayer. The entire system is then immersed in pure buffer and excess vesicle materials are

washed away. This produces a system of addressed bilayer patches where the chemistry of each has been completely controlled.

Selective incorporation of a protein receptor site into the patterned membranes confirms the viability of spatially addressed bilayers as biological sensor devices. To demonstrate this we employed the biotin-streptavidin system. Biotin is a small molecule that can be covalently attached to the headgroup of phosphatidylethanolamine lipids and binds the aqueous protein, streptavidin, with a high affinity. This example not only demonstrates the high level of binding specificity of streptavidin to fluid supported membrane patches containing the biotin receptor site, but also indicates a high level of resistance of fluid phosphatidylcholine membranes to non-specific protein binding.

5.8 EXAMPLE 8 – DESIGN OF BILAYER COATED MICROCHANNELS

In order to design immunosensors and competitive binding assays, it is necessary to address both the aqueous solution as well as the supported bilayer. To achieve this goal we have conducted preliminary experiments on linear arrays of channels that were lithographically patterned into PDMS molds before being placed on planar silica supports. The molds were rendered hydrophilic by treating them with an oxygen plasma for two min (Delamarche *et al.*, 1997). The hydrophilic surfaces of both the PDMS and the glass assisted the initial flow of vesicle solution into the channels. An array of eight channels was made into which egg phosphatidylcholine vesicles containing 1% fluorescently label lipids were separately injected. Each channel was 50 μm wide by 40 μm high and separated from its neighbor by a 25 μm partition. After the bilayers were formed, the channels were flushed with buffer to remove excess vesicles followed by the injection of varying amounts of a red dye into each of the eight channels.

5.9 EXAMPLE 9 – MICROFLUIDIC ASSAY DESIGN

The present invention provides platforms that will facilitate rapid, accurate and convenient measurements of binding constants in *a two-dimensionally fluid environment.* There have only been a few previous examples of microfluidic techniques used to determine binding affinities and these measurements were made serially and homogeneously (Chiem and Harrison, 1998; Duffy *et al.*, 1999). Here an integrated immunoassay for the measurement of binding avidity of multivalent ligand receptor interactions has been designed

employing the biosensors of the present invention. These methods described below have the advantages of rapid multiplexed data collection, high selectivity, and improved accuracy.

To obtain a binding constant for an individual set of ligand-receptor binding conditions, we will exploit the polydimethylsiloxane channels described above. Dry microchannels are created by contacting patterned PDMS with clean glass supports. Aqueous solutions easily flow into these channels when the surface of the PDMS has been plasma treated to render it hydrophilic. Upon vesicle injection, the floor of the channel is coated with a solid supported lipid bilayer. In addition, the PDMS walls and ceilings become coated with bilayers as well. This polymer supported phospholipid material appears to be fluid, but more significantly it resists nonspecific binding of proteins just as glass supported bilayers do. This is in contrast to untreated PDMS where IgG antibodies, streptavidin, BSA and other proteins are adsorbed to a very high extent (Delamarche *et al.*, 1997).

Hydrophilic channels can be modified with lipid bilayers containing membrane bound ligands by injecting vesicle solutions containing the appropriate ligand constituent into each channel followed by washing with buffer. At this point protein solutions can be flowed through the channels and binding observed. Since a binding curve requires approximately ten to fifteen data points plus an equal number of control channels, about two dozen channels will be arrayed side by side to make such measurements. The control membranes, which are made from bilayers without any binding ligands, are used for monitoring nonspecific protein adsorption at each solution phase protein concentration (Linderman, 1993). Studies suggest that it requires 4-5 min. to inject vesicle solutions into two-dozen channels. Once the membrane-coated channels are prepared, viral, bacterial, as well as protein analytes can be delivered to every channel by microcapillary injection. It should be possible to deliver a unique concentration of analyte to two-dozen channels in approximately 4-5 min.

To substantially speed up the delivery process, laminar flow arraying devices may also be employed using the disclosed biochip devices and apparatus. This allows automatic distribution of an analyte solution at various concentrations into neighboring channels from a single source. The design works by exploiting high-precision diffusion controlled mixing of nanoliter quantities of the protein of interest with buffer inside the bilayer coated microchannels. Due to the very small diameter of the channels employed (50-500 μm), two streams of liquid can flow side by side without turbulent mixing because the Reynolds

numbers inside the channels are typically less than 100 (Cremer and Yang, 1999). As the mixing solutions flow down stream they are divided into a linear array of independent branches, each of which contains a successively higher concentration of the protein. Since the protein concentration is arrayed by simple diffusion, a binding curve for aqueous analytes is obtained in a matter of seconds.

5.10 EXAMPLE 10 – MICROARRAY BASED ASSAYS

Here phospholipid bilayers are employed to create spatially addressed arrays of fluid membrane-based sensors. Planar biochips containing arrays of varying bilayer composition are placed inside a sealed cell containing inlet and outlet tubes. Proteins, viruses, or other analytes can then be introduced by flowing the appropriate biological fluid. Analyte identification and quantification may then be made by exploiting the principles of "electronic nose" based detection (Bartlett, 1999). This method operates by using a series of sensor elements, each of which has a different affinity for the analyte. The pattern of responses generated is then correlated to the nature of the analyte(s) and its (their) concentration(s). This approach is ideally suited for weak interactions such as those between carbohydrate moieties and protein coated viruses (Seto, 2000) where the binding constants for individual interactions are only in the millimolar range. In such cases specificity is conveyed through multivalent interactions (Burke *et al.*, 2000). Subtle differences in binding between various analytes can then be distinguished by the binding pattern generated. For example, all influenza viruses display the receptor protein, hemagglutinin, on their outer surface. This protein binds to sialic acid residues on cell surfaces with an apparent dissociation constant of approximately 2.8 mM for a single ligand-receptor interaction (Sauter *et al.*, 1989). The different influenza viruses, however, have subtle differences in their hemagglutinin. This is most profoundly manifest in a group of variable residues that surround the sialic acid binding pocket (Voet and Voet, 1995). The differences in these peripheral residues should indeed be substantial enough to discriminate amongst different strains of the virus.

One of the most powerful advantages of this microarray approach is the ease with which it permits running one or more controls in parallel with one or more samples of interest, a feature that should lead to an overall increased sensor selectivity. Furthermore, by virtue of running many experiments in parallel, all membranes are exposed to the identical temperature, solution phase pH, ionic strength, and analyte concentrations simultaneously.

5.11 EXAMPLE 11 – SCREENING LARGE NUMBERS OF ANAYLYTES USING BIOSENSORS

This example describes use of the disclosed compartmentalized lipid biosensor arrays for the rapid and simultaneous screening of large numbers of selected analytes. These biosensors can be used for assaying pluralities of discreet analytes on the order of 10^2 to 10^6 aqueous samples or more. In fact, the primary limiting factors controlling the number and arrangement of the compartmentalized lipids are practical ones involving the overall size of the biochip itself, the miniaturization of the discreet compartments on a given biochip, and the practical considerations of engineering scale-up into appropriate commercially-relevant biolipid array systems and devices. Because the disclosed microarray systems employ addressable, compartmentalized biomembranes comprising lipid membranes and discreet aqueous solutions disposed thereon, the technology has broad utility in screening libraries of reagents for a given activity, evaluating candidate drugs, biomimetics, and small molecule activators and/or inhibitors for their ability to interact, inhibit, or activate one or more biochemical events when in contact with a fluid biomebrane in an aqueous environment.

As such, one important use of the disclosed methods is in the fabrication of biolipid microarrays for the purpose of screening for compounds that prevent attachment to a cell membrane. For example, screening a library of pathogens or pathogen-derived samples to identify compounds that either prevent, or cause attachment of such pathogens to one or more animal cells. In an important embodiment, the ability to screen viral, bacterial, microbial, or fungal samples to identify compounds that block attachment to animal cells represents a significant breakthrough in antimicrobial therapies and treatment.

Pathogenesis commences when ligands presented on the pathogen recognize and bind to multiple receptors in the cell membrane. Drugs that interfere with this ligand-receptor attachment constitute chemotherapeutic routes for use against a plethora of diseases including: AIDS, leukemia, and the common flu (Memmen *et al.*, 1998). Unfortunately, this route is largely ignored because the assays currently available do not allow for rapid screening of large numbers of molecules in a biologically relevant setting (*i.e.*, in a two-dimensional fluid membrane). The present technology, however, can be used to *simultaneously* monitor the effectiveness of large numbers of candidate inhibitors to prevent surface attachment events because it exploits surface-supported phospholipid bilayers.

Bilayer fluidity is crucial because receptors and ligands must be able to reorganize through latteral diffusion to optimize binding and/or transduce signals across the membrane of pathogen and/or target (Memmen *et al.*, 1998). Attachment of most pathogens to the host cell is polyvalent in nature, relying on multiple ligand-receptor interactions. Therefore, it is now possible to test efficacy of new drugs or small molecule inhibitors or activators in an environment that permits polyvalent interactions between host and pathogen on a targeted cell surface that remains fluid.

Two types of assays using the disclosed biosensor devices are particularly useful in these regards. In the first, large numbers of compounds may be tested against a single target protein (E-selectin) in a static (non-fluid) assay in which only the affinity of ligand for target is measured. These assays combine the high-throughput nature of a combintorial assay, with the fluid nature of the lipid bilayer. Additionally, the technology offers a simpler, safer, and more homogenous testing regime than many of the other assays presently available. Indeed, by incorporating the appropriate cellular receptor(s), supported bilayers bind ligands in an environment quite similar to the one available *in vivo*, because the membrane-based sensor and its components remain mobile on the test surface (Sackmann, 1996). Furthermore, the technology allows for greater customization, because the concentration of receptor, ligand, lipid composition of the membrane, pH of the environment, salts and metal ion concentrations as well as the temperature of the studies can all be varied simultaneously or independently to permit the assay to be performed under conditions that mimic physiological conditions (*e.g.*, by modulating the pH of the assay, one can approximate conditions in an animal's bloodstream (*i.e.* pH 7.4) or in the stomach (*i.e.* pH 2).

In the biosensor arrays described herein, the lipid (membrane) component(s) is free to move within the confines of a single surface region (*e.g.*, a compartment), but it is not able to cross over into neighboring compartments on the biochip. This strategy allows for fabrication of hundreds, thousands or even millions of fluid phospholipid membranes all arranged in unique addressable discreet compartments arrayed onto a single biochip device, which may be on the order of approximately 1 cm^2. The simultaneous or sequential use of pluralities of biochip devices increases even further the number of individual lipid compartments that may be contacted with a target analyte. The creation of massively parallel assays employing one or more such biochip devices, either alone, or in combination with robotic manipulation, or microarraying methodologies permits a rapid, and economical

means for analyzing large numbers of samples in a convenient biochip apparatus. Furthermore, by employing such biochips in computerized apparatus, the gathering and storage of large volumes of assay data obtained from each such lipid compartment, efficient automated systems may be readily developed. FIG. 7, FIG. 8, and FIG. 9 schematically demonstrate the ability of the present invention to be incorporated into a computerized system, and to provide raw and/or analyzed data to be assembled and stored using computer network, and programmed storage medium capabilities.

The present invention also provides important technological breakthroughs that facilitate high-throughput assay systems of target analytes that activate the binding of one or more other molecules to a lipid bilayer. In this case each compartmentalized aqueous sample in contact with a lipid membrane may comprise unknown molecules (*e.g.*, unique chemical constituents including multifarious lipids, proteins, peptides, receptors, small molecules, and such like) in combination with one or more compounds known to bind to the employed lipid membrane. Target molecules can then be identified in those compartments where the binding of the known compound to the lipid bilayer is increased, augmented, or enhanced. Conversely, the biosensor devices can be used to identify target analytes that *decrease* or *prevent* the binding of one or more known molecules to the lipid bilayer. In such an embodiment, target molecules could be identified in those compartments where the binding of the known compound to the lipid bilayer was decreased, minimized or prevented.

As an example of uses of the present devices and apparatus, one may first select an appropriate cell surface receptor to be investigated (*e.g.*, lipids bearing sialic acid that are recognized by the influenza virus). The selected receptor is then incorporated into phospholipid vesicles through standard methodologies. Receptor-containing vesicles are fused at the liquid-solid interface to an optically-transparent, biomembrane-compatible, substantially planar biochip surface to form a continuous supported phospholipid membrane. The chip is then placed in intimate contact with a lithographically fabricated polydimethylsiloxane mold to form a plurality of identical aqueous compartments depending upon compartment dimensions (Xia and Whitesides, 1998). The length, width, and height of the compartments can be varied depending upon desired parameters. A different inhibitor is then added to each compartment typically using an array of capillary tips or needles to transfer different solutions from a master to each test site simultaneously (Schena *et al.*, 1995). Following this, all compartments are exposed to an identical concentration of

particular pathogen. Surface binding of the pathogen to bilayer-bound receptor is then monitored with surface specific optical microscopy; either total internal reflection fluorescence (Axelrod *et al.*, 1984), or surface plasmon microscopy (Frutos and Corn, 1998). In the former case, the study is conducted on an optically transparent biochip such as borosilicate and the pathogen is fluorescently labeled. Surface bound fluorophores are excited *via* an evanescence wave from a laser beam undergoing total internal reflection through a dove prism. Alternatively, the need for fluorescently labeling the pathogen can be avoided by performing the experiment in a surface plasmon microscope. In this case a membrane compatible Au film or other suitable material is then substituted for the borosilicate support.

Antibody Detection Systems:

In an exemplary antibody system dinitrophenyl (DNP)-substituted lipids and anti-DNP antibody have been tested using soluble DNP-glycine as a competitor (drug mimic).

Anti-Arthritis System:

In the third system, an anti-arthritis assay, the receptor protein E-selectin was incorporated into a lipid bilayer, and a mold in which the aqueous compartments are designed to be long channels through which reagents and buffers can be flowed was employed. White blood cells and drug candidates are delivered to one end of the channel and put under flow conditions by continuously pumping ligand in buffered saline. Leukocyte adhesion is monitored with optical and fluorescence microscopy. This system illustrates an important advantage of the present technology: massively parallel assays for the simultaneous measurement of polyvalent surface absorption events under physiological flow conditions with a library of drug candidates.

5.11.1 TIRFM AND SPM MEASUREMENTS

To quantitatively determine the amount of surface bound analyte in the channels (or microwells) while discriminating against free species, fluorescently tagged analytes and total internal reflection fluorescence microscopy (TIRFM) may be employed (Alexrod *et al.*, 1984). TIRFM is a surface specific technique capable of exciting fluorophores that are surface bound rather than free in the bulk solution. TIRFM is surface selective because the evanescence field generated by the total internal reflection of a beam of light decays exponentially as the field penetrates into the adjacent medium. The substrate beneath the

patterned surface on which the bilayer resides can be made of optically transparent float glass, which has an index of refraction of 1.50. Above this surface is the aqueous phase with an index of refraction close to 1.33. When a laser beam is incident on this interface at approximately 79°, the intensity of the evanescence field falls to 37% (1/e) of its initial value at a distance of 75 nm above the liquid-solid interface for 594-nm radiation. The relevant equation is:

$$(1) \qquad I(z) = I_0 e^{-z/d} \quad \text{where} \quad d = \frac{\lambda_0}{4\pi}[n_1^2 \sin^2\theta - n_2^2]^{-1/2}$$

where I is the intensity of the incident light beam, z is the distance from the interface measured in the normal direction, n_1 is the index of refraction of medium 1 (high index medium from which the light is incident) and n_2 is the index of refraction of medium 2 (low index medium into which the evanescent field is propagating), θ is the angle that the radiation makes with the surface normal and λ_0 is the wavelength.

In real world applications it is, of course, not possible to label analytes with fluorescent tags before they are flowed over a biosensor. It will therefore be necessary to employ a surface specific detection method that allows unaltered samples to be monitored on the platforms described herein. One such technique is surface plasmon microscopy (SPM). The SPM technique operates on the underlying principles of surface plasmon resonance (SPR) spectroscopy. SPR, a process which has been described elsewhere in great detail (Liedberg and Johansen, 1998; Jung *et al.*, 1998), employs an external light source to drive the collective oscillation of electrons in a free electron metal film, causing an optical wave to propagate along the interface between the metal and the neighboring environment. Because the field created adjacent to the metal is an evanescent wave, it also falls off exponentially with distance (just like in fluorescence). It is this evanescence field near the surface of the metal that is used as the basis for surface sensitive detection in SPR. Indeed, even a small change in the near surface index of refraction of the material in contact with the metal surface will affect the plasmon oscillations. This disturbance is measured as a change in the angle of maximum attenuation of the reflected signal.

Using a well-collimated beam of light from a monochromatic source at a fixed angle enables spatial information to be obtained from a planar SPR sample. To accomplish this, a laser beam is telescoped out to the desired diameter and then reflected off the sample (Frutos and Corn, 1998). Detecting the reflected beam with a standard CCD camera then provides

spatial resolution. Literature results report resolution down to a few microns with excellent signal to noise (Schmitt and Knoll, 1991). Thus by using patterned samples, the SPM can be employed as an array detector for monitoring binding kinetics at hundreds or even thousands of chemically distinct surface sectors simultaneously. In order to use an SPM based sensing device, an appropriate conducting substrate must be rendered compatible with the fluid lipid bilayer platforms. This is readily accomplished by derivatizing a gold surface with mercaptoethanol or other thiols that terminate with alcohol or acid moieties.

5.11.2 Data Fitting and Processing

To quantify the strength of ligand receptor interactions in the systems described in above, curves can be plotted for surface analyte coverage *vs.* bulk phase concentration to obtain binding constants. The ligand concentration on the surface (number density of ligands presented) is held constant while the solution concentration of the receptor species is varied (Stewart and Steensgaard, 1983). The fraction of bound ligand (bound/total) can then be plotted as a function of receptor concentration. This is typically done for ten to fifteen different concentrations of the soluble receptor and graphed on a semi-log plot.

The common feature of most binding curves is the "S" shape that is depicted in the figure. In the case of multivalent ligand-receptor binding the precise shape can vary since the binding process can be positively cooperative, noncooperative, or negatively cooperative (Lauffenburger and Linderman, 1993; Mammem *et al.*, 1995; Hughes, 1992; Gennis, 1989). In the case of positively cooperative binding, the S shape rises sharply over a narrow concentration range as the first binding events quickly induce more. On the other hand, negatively cooperative binding causes the S shape to flatten, while noncooperative binding gives rise to curves of intermediate shape. In the case of soluble multivalent receptors binding to membrane-associated ligands, the binding is usually considered to be negatively cooperative as the rearrangements and bindings after the initial binding event are typically less favorable (Mammem *et al.*, 1998; Pisarchick *et al.*, 1992). Detailed references are available for data fitting (Stewart and Steensgaard, 1983). Langmuir isotherms, Scatchard plots, and more sophisticated models that take explicit account of the mechanistic details of the binding process may also be employed.

5.X EXAMPLE X – FABRICATION OF PHOSPHOLIPID BILAYER COATED MICROCHANNELS FOR ON-CHIP IMMUNOASSAYS

The present example describes a new class of microfluidic immunoassays based upon solid supported lipid bilayers. Two-dimensionally fluid bilayer material, which can accommodate multivalent binding between surface bound ligands and aqueous receptors, was coated on the surface of polydimethylsiloxane microchannels. The bilayers contained dinitrophenyl (DNP) conjugated lipids for binding with bivalent anti-DNP antibodies. Twelve independent data points of surface coverage versus bulk protein concentration could be made simultaneously by forming a linear array of channels and flowing fluorescently labeled antibodies into them. This enabled an entire binding curve to be obtained in a single experiment. The measured apparent binding constant for the DNP/anti-DNP system was 1.8 μM. The methodology for performing heterogeneous assays developed here not only produces rapid results, but also requires much less protein than traditional procedures and eliminates some standard sources of experimental error.

A multivalent immunoassay has been designed for monitoring the binding of bivalent anti-dinitrophenyl antibodies to phospholipid bilayer surfaces containing dinitrophenyl haptens. The DNP/anti-DNP system was chosen because it is well characterized with phospholipid bilayers (Thompson *et al.*, 1993; Pisarchick and Thompson, 1990; Pisarchick *et al.*, 1992; Timbs *et al.*, 1991; Balakrishnan *et al.*, 1982; Cooper *et al.*, 1981). The assay was fully incorporated into a microfluidic network where a dozen concentration *vs.* coverage data points were obtained simultaneously in a highly accurate, rapid format requiring only minimal amounts of protein analyte. Surface specificity was achieved by using total internal reflection fluorescence microscopy. This technique allowed the surface coverage to be monitored in the presence of high concentrations of bulk analyte.

5.X.1 MATERIALS AND METHODS

All immunoassay studies were conducted inside linear arrays of bilayer coated microchannels. The channels were formed by placing lithographically patterned polydimethylsiloxane (PDMS) molds (Dow Corning Sylgard Silicone Elastomer-184, Krayden, Inc.) into conformal contact with planar borosilicate coverslips (Xia and Whitesides, 1998; Delamarche *et al.*, 1997). The molds were made by curing the low molecular weight polymer with a cross linker on a photolithographically patterned master prepared by exposing and developing a photoresist patterned surface. The elastomeric mold,

which bore the negative pattern of the master, was carefully peeled off and washed repeatedly with acetone and ethanol. Before contact was made with the glass substrate, the PDMS surface was rendered hydrophilic by oxygen plasma treatment for 15 sec (plasma cleaner PDC-32G, Harrick Scientific, Ossining, NY). The glass coverslips employed in these studies were cleaned in hot surfactant solution (ICN x7 detergent, Costa Mesa, CA), rinsed at least 20 times in purified water from a NANOpure Ultrapure Water System (Barnstead, Dubuque, IA) and then baked in a kiln at 400°C for 4 hr. before use.

Once the hydrophilic microchannels were formed, buffer solutions containing approximately 1 mg/ml concentrations of small unilamellar vesicles (SUVs) were injected into the channels through 500 μm holes, which were patterned into each end of each channel as inlet and outlet ports. Supported bilayers formed spontaneously on the channel surfaces via the vesicle fusion method (Brian and McConnell, 1984). Excess lipid vesicles were then flushed from the microchannels by flowing pure buffer solution until the channels were judged to be clear of free vesicles by epifluorescence microscopy. All supported membranes primarily consisted of egg phosphatidylcholine (Egg PC, Avanti Polar Lipids, Alabaster, AL) doped with a small concentration of phosphatidylethanolamine lipids conjugated with dye groups and haptens covalently attached at the free amine. The dye labeled lipids, Texas Red DHPE and fluorescein-DHPE, were purchased from Molecular Probes, Eugene, OR. SUVs were formed by standard procedures (Barenholz *et al.*, 1977). Briefly, mixtures of lipids, probes, and haptenylated lipids were mixed in chloroform and added to a clean round bottom flask. The chloroform was then evaporated under a stream of dry nitrogen followed by further drying for two hours under vacuum. The dried lipids were reconstituted in the desired buffer solution to a concentration of 1.7 mg/ml. Small unilamellar vesicles were formed by probe sonication of the suspension to clarity followed by centrifugation at 38,000 rpm for 30 min. to remove titanium particles. The supernatant was then centrifuged again at 52,000 rpm for 3 hr. to remove large lipid aggregates. The SUVs were stored at 4°C before use.

Membranes containing N-dinitrophenylaminocaproyl phosphatidylethanolamine (DNP-Cap PE, 16:0), which was also purchased from Avanti, were used in the ligand-receptor binding studies. Polyclonal anti-dinitrophenyl-KLH IgG antibody was purchased from Molecular Probes and labeled with Alexa Fluor 594 using a standard antibody labeling kit (A10239, Molecular Probes). Labeling the antibody yielded 2.4 fluorophores/protein as

judged by UV absorption spectroscopy. The dye-labeled protein was concentrated to 13.2 μM by evaporation of water in an evacuated desiccator. The material was stored in a pH 7.2 buffer solution containing 0.01M sodium phosphate, 0.15M NaCl, and 0.2 mM sodium azide before use.

Images of the supported membranes and antibodies in the microchannels were obtained by epifluorescence microscopy (E800 fluorescence microscope, Nikon) using a x10 objective. Total internal reflection experiments were performed to discriminate between antibodies in solution and those bound to the surface (Axelrod *et al.*, 1984). These studies were conducted by reflecting a 1 mW 594 nm helium neon laser beam (Uniphase, Manteca, CA) off the sample surface through a dove prism optically coupled to the planar coverslip surface through immersion oil. The laser beam was telescoped out with a x5 lens set to create an incident intensity profile that varied less than 3% across the width of the microchannel array.

All images were captured with a Photometrics Sensys CCD Camera and stored using Metamorph software from Universal Imaging Corporation. The images were then transferred to Adobe Photoshop and processed using false color imaging techniques.

5.X.2 Results

5.X.2.1 Preparing Bilayer Coated Microchannels

Epifluorescence images was made of an array of eight 50 μm wide microchannels coated with fluorescently labeled SLBs. Each microchannel was addressed individually by injecting SUVs in a10 mM PBS buffer at pH 7.2 into the channel inlet ports. The odd numbered microchannels contained 1 mol% Texas Red DHPE probes in the lipid bilayers, while the even numbered channels were prepared with 3 mol% fluorescein DHPE probes. Vesicle fusion occurred on both the PDMS channel walls (Hovis and Boxer, 2000) as well as on the underlying borosilicate substrate. Fluorescence recovery after photobleaching experiments indicated that the supported membranes were mobile on both materials. It should be noted that vesicle injection was performed in all channels within 3-4 min. after exposing the PDMS mold to the oxygen plasma. This not only ensured that the channels were sufficiently hydrophilic to induce flow by positive capillary action, but also led to the formation of high quality bilayers on the PDMS surface. Uniform formation of the bilayers on the polymer walls as well as the borosilicate substrate was important because it rendered

the entire microchannel surface resistant to nonspecific adsorption of IgG antibodies in subsequent immunoassay studies. Control studies showed that protein adsorption to bilayer-coated channels was suppressed by at least two orders of magnitude in comparison with uncoated channels as judged by quantitative fluorescence measurements. Furthermore, the bilayers appeared to be stable on the microchannels for at least several weeks.

5.X.2.2 Designing a Heterogeneous Binding Assay

In order to obtain sufficient data for quantitative measurements of ligand-receptor binding as a function of antibody concentration, measurements were made over two orders of magnitude in protein concentration. Twelve microchannels were arrayed on a single chip. Each channel was injected with a solution of small unilamellar vesicles composed of 92 mol% egg PC, 5 mol% DNP-PE and 3 mol% fluorescein DHPE in a 10 mM PBS buffer at pH 7.2. After flushing out excess vesicles, the channels were imaged by epifluorescence microscopy. At this point, various concentrations of Alexa dye labeled anti-DNP were injected into the channels, with the lowest protein concentration on the left side and the highest on the right. Because surface binding causes bulk concentration depletion, protein solution was flowed continuously through the channels until the bulk concentration became stabile. This required an aliquot roughly equal to 4 or 5 times the channel volume in the channels with the lowest protein concentration, while the highest concentration channels required considerably less flow, as expected. A line profile generated from the epifluorescence image of these channels was obtained. The majority of the signal emanated from the bulk.

To obtain information on surface coverage, it was necessary to discriminate between surface bound antibodies and the vastly larger population in the bulk solution. This was achieved by total internal reflection fluorescence microscopy (TIRFM) (Axelrod *et al.*, 1984). TIRFM creates an evanescence wave that decays as a function of distance from the surface as:

$$I(z) = I_0 e^{-z/d} \quad \text{and} \quad d = \frac{\lambda_0}{4\pi}[n_1^2 \sin^2\theta - n_2^2]^{-1/2}$$

where I is the intensity of the incident light beam, z is the distance from the interface measured in the normal direction, n_1 is the index of refraction of medium 1 (high index medium from which the light is incident) and n_2 is the index of refraction of medium 2 (low index medium into which the evanescent field is propagating), θ is the angle which the

radiation makes with the surface normal and λ_0 is the wavelength. The substrate beneath the channels on which the bilayers resided was made of borosilicate float glass, which has an index of refraction of about 1.52. Inside the channels above this surface was the aqueous phase with an index of refraction close to 1.33. Since the laser beam was incident on this surface at approximately 79°, the intensity of the evanescence field fell to 37% (1/e) of its initial value at a distance of 70 nm above the liquid/solid interface for the 594 nm radiation employed.

The same system was also imaged by TIRFM. The fluorescence intensity was no longer linear with respect to bulk concentration. The signal, however, represented a combination of specifically bound antibody, nonspecifically bound antibody, and near surface antibody in the bulk solution. To discriminate against signal arising from the latter two effects, the study was repeated under the identical conditions, but in the absence of DNP-PE lipids in the SLBs. In this case, the fluorescence intensity from the total internal reflection studies was dramatically weaker as demonstrated by the line profile. After flowing the antibody out of the channels, the TIRFM image was repeated and the signal fell by more than an order of magnitude with respect to the earlier results. This last study demonstrated that most of the background signal arose from either near surface bulk proteins or from rather weakly bound surface species rather than from irreversibly adsorbed IgG. A binding curve for coverage *vs.* bulk protein concentration was obtained for the DNP/anti-DNP system by subtracting the background from the data obtained, and was plotted on a semi-log scale. The half coverage point on the curve occurs at a concentration of 1.8 μM and is in good agreement with data from previous studies (Pisarchick and Thompson, 1990; Mammem *et al.*, 1995).

An important feature of the SLB/microfluidic techniques shown here is that all data points are collected simultaneously. In addition to merely being more rapid than assays that obtain data sequentially, the measurements should also be more accurate, as sources of error such as changes in flash lamp intensity over successive measurements are eliminated. These methods also use a smaller quantity of protein than previous techniques; however, measurements of binding curves in heterogeneous assays require more analyte than sensors designed merely to detect the presence of such species. The volume of protein solution used to fill the microchannels in these experiments was approximately 2 μL when the injection port volume is included. Furthermore, it was necessary to flow excess protein solution

through each channel to account for bulk depletion by surface adsorption. In total, approximately 35 μg (230 picomoles) of IgG antibody was used to perform a single multivalent binding constant measurement. This amount can be reduced by an order of magnitude or more by using smaller channels, syringes, and injection ports.

5.12 EXAMPLE 12 --PIN REPLICATOR AND MACROSCOPE DESIGN

One drawback of microcapillary injection methods is the time consuming process that is needed for manually addressing large arrays of supported membranes into each of the individual sectors. The procedure involves the delivery of vesicle solutions to a photopatterned chip from a master array of well plates that is pre-addressed with vesicle solutions. The solutions are transferred simultaneously by using an array of flat tipped pins (also called a pin replicator). The advantages of this approach are substantial. One master well plate can be produced manually or by inkjet printing (Lemmo *et al.*, 1997) and used hundreds or even thousands of times. Membrane libraries are simply created once and stored for future use. Such well plates and their associated pin replicators come in standard formats such as 96, 384, 1536, and 6144 microwells.

Applying this technology to supported membrane architectures is straightforward. The only caveat of switching to standardized well plates is one of size. All standard well formats are embedded on rectangular grids which are roughly 3.5" × 5". This is not a problem in terms of fabrication; however, the area that can be observed under an epifluorescence microscope is usually restricted to a few square millimeters at most. Unfortunately, even the high-density 6144 well format is made of wells that are 1.125 mm apart. Therefore, in order to observe larger fields of view a macroscope is the preferred method of viewing (Ratzlaff and Grinvald, 1991). Macroscopes use considerably lower magnification (*e.g.*, x0.3, x0.5, x1) than microscopes (*e.g.*, x4, x10, x100) thus allowing much larger areas to be imaged. Although low magnification objectives can be fitted on traditional microscopes, they usually suffer from a greatly reduced numerical aperture (light gathering power). By contrast, macroscopes are designed to accommodate much larger collecting lenses than conventional microscopes and, hence, preserve the large numerical apertures necessary to perform the fluorescence imaging experiments described herein. For example a standard macroscope design permits imaging a 2 cm × 2 cm sample area that contains roughly 350 bilayer patches in a single short exposure.

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While the disclosed systems of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the disclosed system and in the steps or in the sequence of steps described herein without departing from the concept, spirit and scope of the invention. All such similar modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims. Accordingly, the exclusive rights sought to be patented are as described in the claims below.

CLAIMS:

1. A lipid microarray device comprising:

(a) a substantially planar substrate;

(b) a plurality of independently addressable isolated lipid bilayers partitioned discreetly upon said substrate;

(c) at least a first aqueous solution disposed upon at least a first of said independently addressable partitioned lipid bilayers; wherein said at least a first aqueous solution comprises at least a first constituent that interacts with said lipid bilayer, and

(d) at least a second aqueous solution disposed upon at least a second of said independently addressable partitioned lipid bilayers; wherein said at least a second aqueous solution comprises:

(i) said at least a first constituent that interacts with said lipid bilayer and at least a second constituent that binds to said first constituent or to said lipid bilayer or;

(ii) at least a second, distinct constituent that interacts with said lipid bilayer.

2. The device of claim 1, wherein said plurality of independently addressable isolated lipid bilayers are partitioned discreetly upon said substrate by contacting said substrate with a mold, said mold comprising a plurality of indentations, such that a seal is formed between said mold and said substrate, thereby forming said discreetly partitioned independently addressable isolated lipid bilayers upon said substrate.

3. The device of claim 1 or 2, wherein said plurality of independently addressable isolated lipid bilayers are comprised of essentially the same biological components.

4. The device of claim 1 or 2, wherein said plurality of independently addressable isolated lipid bilayers are comprised of essentially distinct biological components.

5. The device of any preceding claim, further comprising an exogenous component.

6. The device of claim 5, wherein said exogenous component comprises a lipid, a lipid bilayer component, or an aqueous constituent.

7. The device of any preceding claim, further comprising a detectable label.

8. The device of claim 7, wherein said plurality of independently addressable isolated lipid bilayers further comprises said detectable label.

9. The device of claim 7, wherein said first or said second aqueous solution further comprises said detectable label.

10. The device of claim 7, wherein said first or said second constituent further comprises said detectable label.

11. The device of claim 7, wherein said detectable label is comprised within an exogenous component.

12. The device of any preceding claim, further comprising a detectable label selected from the group consisting of a radioactive, chromogenic, a fluorescent, an epifluorescent, a spin label, and a detectable ligand.

13. The device of any preceding claim, wherein substantially all of said plurality of independently addressable isolated lipid bilayers has disposed above them an aqueous solution that comprises said first constituent.

14. The device of any preceding claim, wherein substantially all of said plurality of independently addressable isolated lipid bilayers has disposed above them an aqueous solution that comprises said second constituent.

15. The device of any preceding claim, wherein substantially all of said plurality of independently addressable isolated lipid bilayers has disposed above them an aqueous solution that comprises an exogenous component.

16. The device of any preceding claim, wherein substantially all of said plurality of independently addressable isolated lipid bilayers comprises an exogenous component.

17. The device of any preceding claim, wherein said plurality of independently addressable isolated lipid bilayers are comprised of essentially the same biological components, and further wherein substantially all of said plurality of independently

addressable isolated lipid bilayers have disposed above them an aqueous solution that comprises said first and said second constituents.

18. The device of any preceding claim, wherein said first and said second constituents are substantially the same in both said first and said second aqueous solutions, and said second aqueous solutions further comprises at least a second constituent.

19. The device of any preceding claim, wherein said first and said second constituents are unique in each of said first and said second aqueous solutions.

20. The device of any preceding claim, further comprising at least a first and a second detectable label.

21. The device of any preceding claim, wherein said plurality of independently addressable isolated lipid bilayers are comprised of essentially the same biological components, and further wherein substantially all of said plurality of independently addressable isolated lipid bilayers have disposed above them an aqueous solution that comprises the same first constituent, and a plurality of second distinct constituents.

22. The device of any preceding claim, wherein said plurality of independently addressable isolated lipid bilayers are comprised essentially of the same biological components and the aqueous solution disposed above each of said bilayers is substantially unique.

23. The device of any preceding claim, wherein each of said aqueous solutions is disposed upon each of said partitioned bilayers by a needle, syringe, micropipette, microcapillary tube or a replicator pin.

24. The device of claim 23, wherein said replicator pin is comprised of a substantially inert material.

25. The device of claim 24, wherein said substantially inert material comprises a plastic, a polymer, a halogenated polymer, glass, quartz, metal, or a ceramic.

26. The device of any preceding claim, wherein each of said aqueous solutions is disposed upon each of said partitioned bilayers in an environment of essentially 100% relative humidity.

27. The device of any preceding claim, wherein said substantially planar substrate comprises a liquid-solid interface for fusing with each of said plurality of independently addressable isolated lipid bilayers.

28. The device of any preceding claim, wherein said substantially planar substrate further comprises a plurality of indentations capable of receiving said plurality of independently addressable isolated lipid bilayers.

29. The device of any preceding claim, wherein said substrate comprises a material selected from the group consisting of borosilicate, silica, derivatized silica, quartz, mica, thiol-functionalized gold, silicon, derivatized silicon, silicon oxide, derivatized

silicon oxide, tin oxide, derivatized tin oxide, indium tin oxide, derivatized indium tin oxide, silanized silica, and $SrTiO_3$.

30. The device of any preceding claim, wherein said plurality of independently addressable isolated lipid bilayers are partitioned discreetly upon said substrate by microcontact displacement, vesicle fusion, mechanical patterning, or etching.

31. The device of claim 2, wherein said mold comprises a substantially elatsomeric material and said substrate comprises a substantially rigid material.

32. The device of any preceding claim, wherein said mold comprises a material selected from the group consisting of a plastic, a polymer, a halogenated polymer, an elastomer, borosilicate, silica, derivatized silica, quartz, mica, thiol-functionalized gold, silicon, derivatized silicon, silicon oxide, derivatized silicon oxide, tin oxide, derivatized tin oxide, indium tin oxide, derivatized indium tin oxide, silanized silica, and $SrTiO_3$.

33. The device of claim 32, wherein said halogenated polymer is polytetrafluoroethylene (Teflon®) or said elastomer is polydimethylsiloxane (PDMS).

34. The device of any preceding claim, wherein said isolated lipid bilayer comprises a lipid component selected from the group consisting of a phospholipid, a cardiolipid, a lysophospholipid, a ceramide, a ganglioside, a cerebroside, a glycolipid, a sterol, a sphingolipid, and a derivatized lipid conjugate.

35. The device of any preceding claim, wherein said isolated lipid bilayer further comprises a non-lipid component.

36. The device of claim 35, wherein said non-lipid component is selected from the group consisting of a protein, a peptide, a glycoprotein, a receptor, an antibody, an antibody fragment, an enzyme, a protease, a lipase, a phosphatase, a kinase, a nucleic acid binding protein, an ion channel, a signal transduction polypeptide, an MHC molecule, a toxin receptor, and a transport protein.

37. The device of any preceding claim, wherein said first or said second aqueous solutions comprises at least a first lipid, carbohydrate, polysaccharide, polypeptide, peptide, nucleic acid, small molecule component, biomimetic, antibody, antigen binding fragment, virus, bacterium, fungus, or eukaryotic cell.

38. The device of any preceding claim, wherein said first or said second aqueous solutions comprises a ligand, a hormone, a growth factor, a neurotransmitter, an adhesion molecule, a viral coat protein, a cell surface protein, a prion, an antigen, an extracellular matrix protein or peptide, a toxin, a cell component of a virus, bacterium, fungus, or eukaryotic cell, or a secretagogue.

39. The device of any preceding claim, wherein said plurality of second distinct constituents are selected from a library that is comprised of combinatorial chemistry products, secondary metabolite products, small molecules, or natural products of animal, plant, microorganismal, viral, soil, marine, or rainforest origin.

40. An apparatus, comprising the device of any preceding claim, and a robotic device capable of spatially addressing each of said plurality of isolated lipid bilayers partitioned discreetly upon said substrate.

41. The apparatus of claim 40, wherein said robotic device is capable of discreetly disposing said first and said second aqueous solutions upon said first and said second independently addressable partitioned lipid bilayers.

42. The apparatus of claim 40 or claim 41, further comprising a program storage medium encoded with instructions that, when executed by a computer, control said robotic device.

43. The apparatus of any one of claims 40 to 42, further comprising a controller programmed with instructions for controlling said robotic device.

44. The apparatus of any one of claims 40 to 43, further comprising a computing system programmed with instructions for controlling said robotic device.

45. The apparatus of any one of claims 40 to 44, wherein said computing system comprises a part of a networked computer system.

46. An apparatus comprising a plurality of the device of any one of claims 1 to 39.

47. The apparatus of claim 46, wherein at least a first and a second of said plurality of devices comprise distinct independently addressable isolated lipid bilayers.

48. An apparatus for constructing the microarray device of any one of claims 1 to 39, the apparatus comprising:

(a) a platform on which a substrate may be positioned; and

(b) means for discreetly partitioning a plurality of independently addressable isolated lipid bilayers upon said substrate.

49. The apparatus of claim 48, wherein the discreet partitioning means comprises a mold, including a plurality of indentations.

50. An apparatus for disposing an aqueous sample onto at least a first discreetly partitioned lipid bilayer of a microarray device, the apparatus comprising:

(a) a platform on which the microarray device may be positioned; and

(b) means for introducing said aqueous sample onto at least a first of said partitioned isolated lipid bilayers.

51. The apparatus of claim 50, wherein said means for introducing said aqueous sample onto at least a first of said partitioned isolated lipid bilayers includes a microarray of replicating pins, said pins capable of independently addressing said plurality of partitioned lipid bilayers.

52. The apparatus of claim 50 or claim 51, wherein said means for introducing said aqueous sample onto at least a first of said partitioned isolated lipid bilayers includes

an array of microcapillary devices, said devices capable of independently addressing said plurality of partitioned lipid bilayers.

53. An apparatus for detecting a target constituent in an aqueous sample contacted with at least a first partitioned isolated lipid bilayer of a microarray device, the apparatus comprising:

(a) a platform on which said device may be positioned when said device contains said aqueous sample and a detectable label; and

(b) means for detecting said detectable label.

54. The apparatus of claim 53, wherein said means for detecting said detectable label includes a detector capable of detecting a radioactive label, a spin label, a chromogenic compound, a fluorescence label, a detectable ligand, or an epifluorescence label.

55. An apparatus for identifying the presence of a target constituent in an aqueous sample disposed upon an independently addressable partitioned lipid bilayer of a lipid microarray device that comprises a detectable label, wherein the presence of said constituent alters the interaction of said aqueous sample and said lipid bilayer, said apparatus comprising:

(a) a platform on which said lipid microarray device may be positioned; and

(b) means for performing at least one of the following functions:

(1) isolating a plurality of independently addressable lipid bilayers into discreet partitions upon said substrate;

(2) introducing said aqueous sample onto at least a first of said partitions, and introducing a detectable label into said partitions; and

(3) detecting said detectable label in said partitions.

56. An apparatus for identifying the presence of a target constituent in an aqueous sample disposed upon an independently addressable partitioned lipid bilayer of a lipid microarray device that comprises a detectable label, wherein the presence of said constituent alters the interaction of said aqueous sample and said lipid bilayer, said apparatus comprising:

(a) a platform on which said lipid microarray device may be positioned; and

(b) means for performing the following functions:

(1) isolating a plurality of independently addressable lipid bilayers into discreet partitions upon said substrate;

(2) introducing said aqueous sample onto at least a first of said partitions, and introducing a detectable label into said partitions; and

(3) detecting said detectable label in said partitions.

57. A method for identifying a constituent that directly or indirectly interacts with at least a first component of a lipid bilayer, from a population of aqueous solutions, said method comprising:

(a) applying said population of aqueous solutions to the device of claim 1; and

(b) identifying a constituent from at least one of said aqueous solutions in said population that directly or indirectly interacts with at least a first component of a lipid bilayer disposed in said device.

5

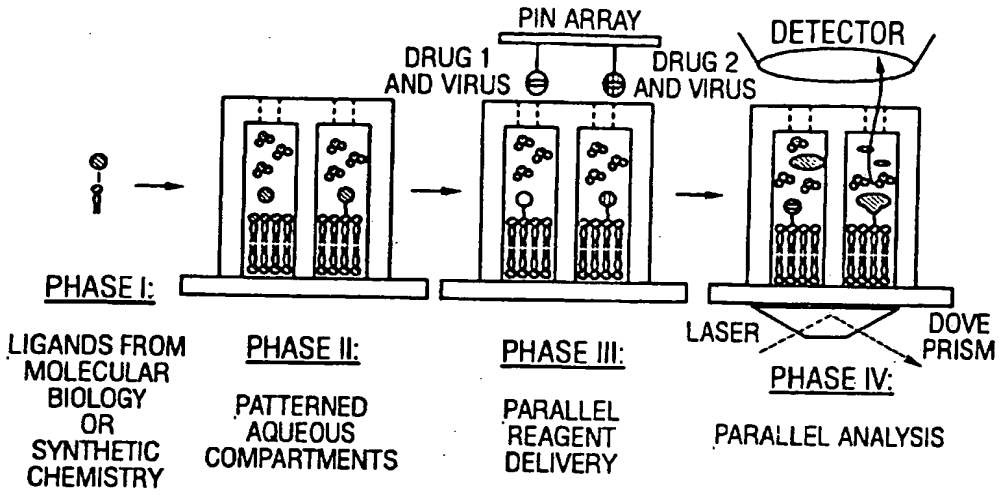


FIG. 1

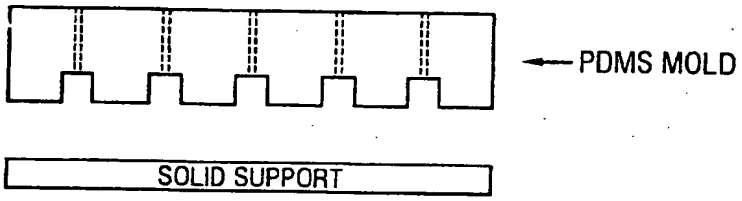


FIG. 2A

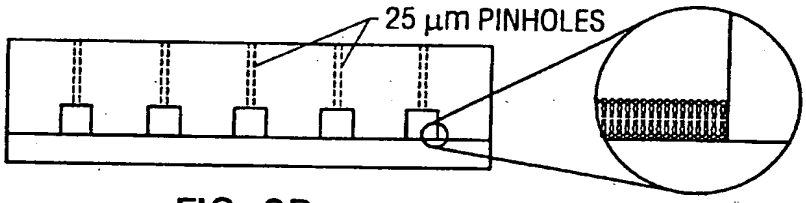


FIG. 2B

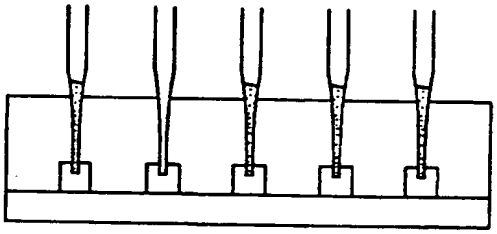


FIG. 2C

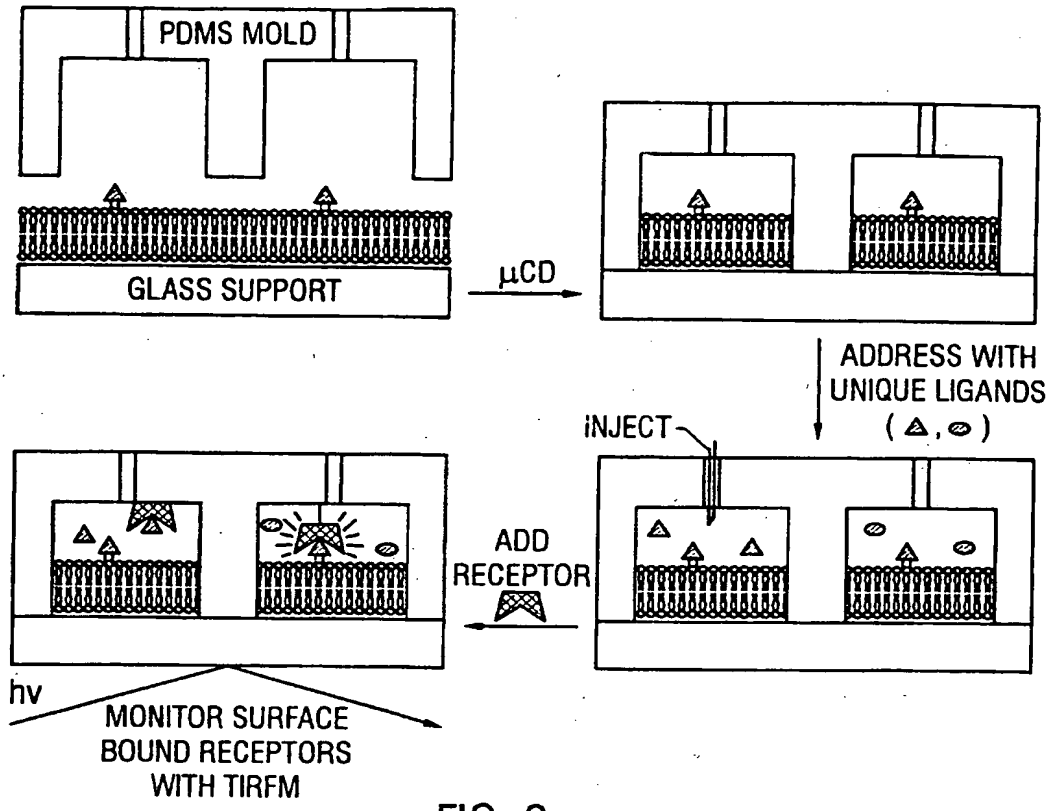

PDMS MOLD
GLASS SUPPORT
μCD
ADDRESS WITH
UNIQUE LIGANDS
(△, ⊘)
INJECT
ADD
RECEPTOR
hv
MONITOR SURFACE
BOUND RECEPTORS
WITH TIRFM

FIG. 3

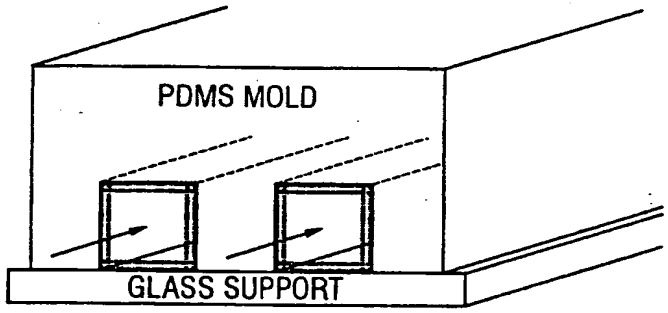

PDMS MOLD
GLASS SUPPORT

FIG. 4

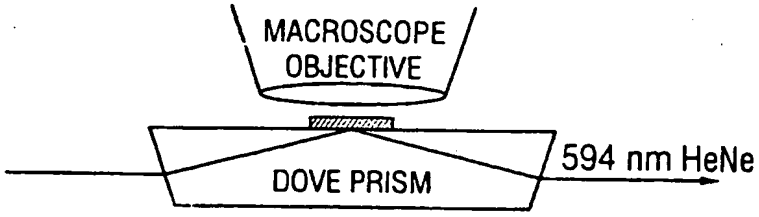


FIG. 5

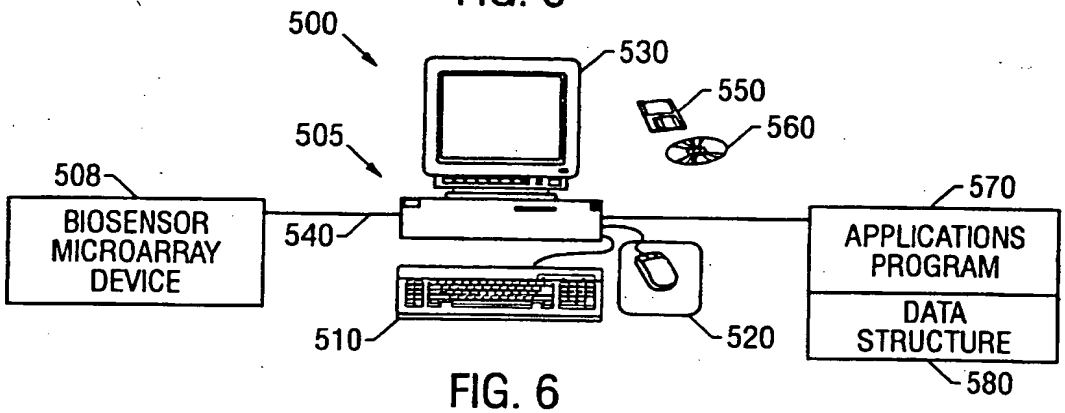


FIG. 6

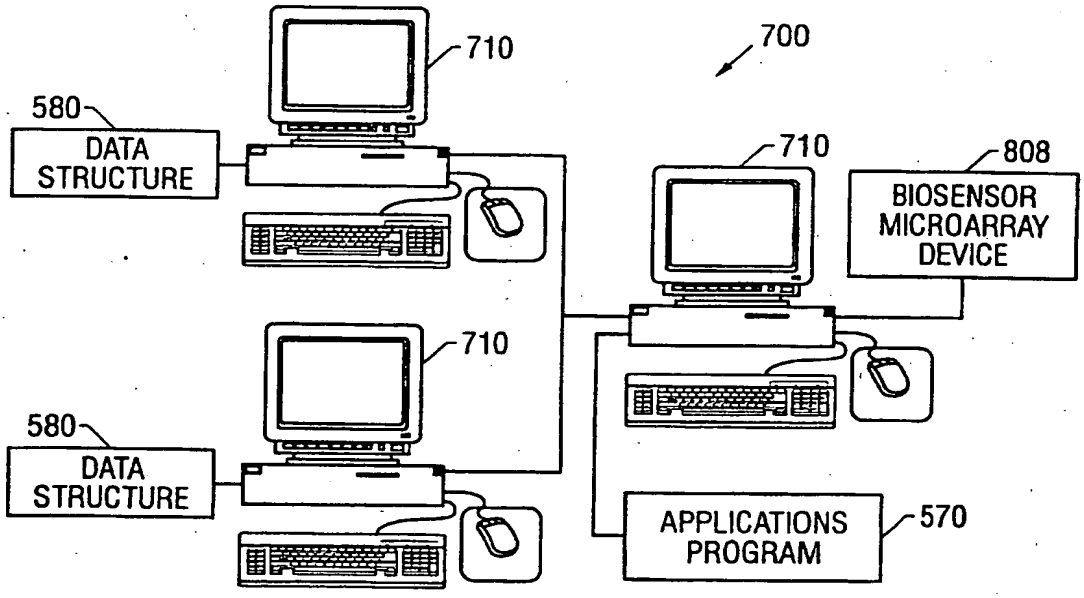
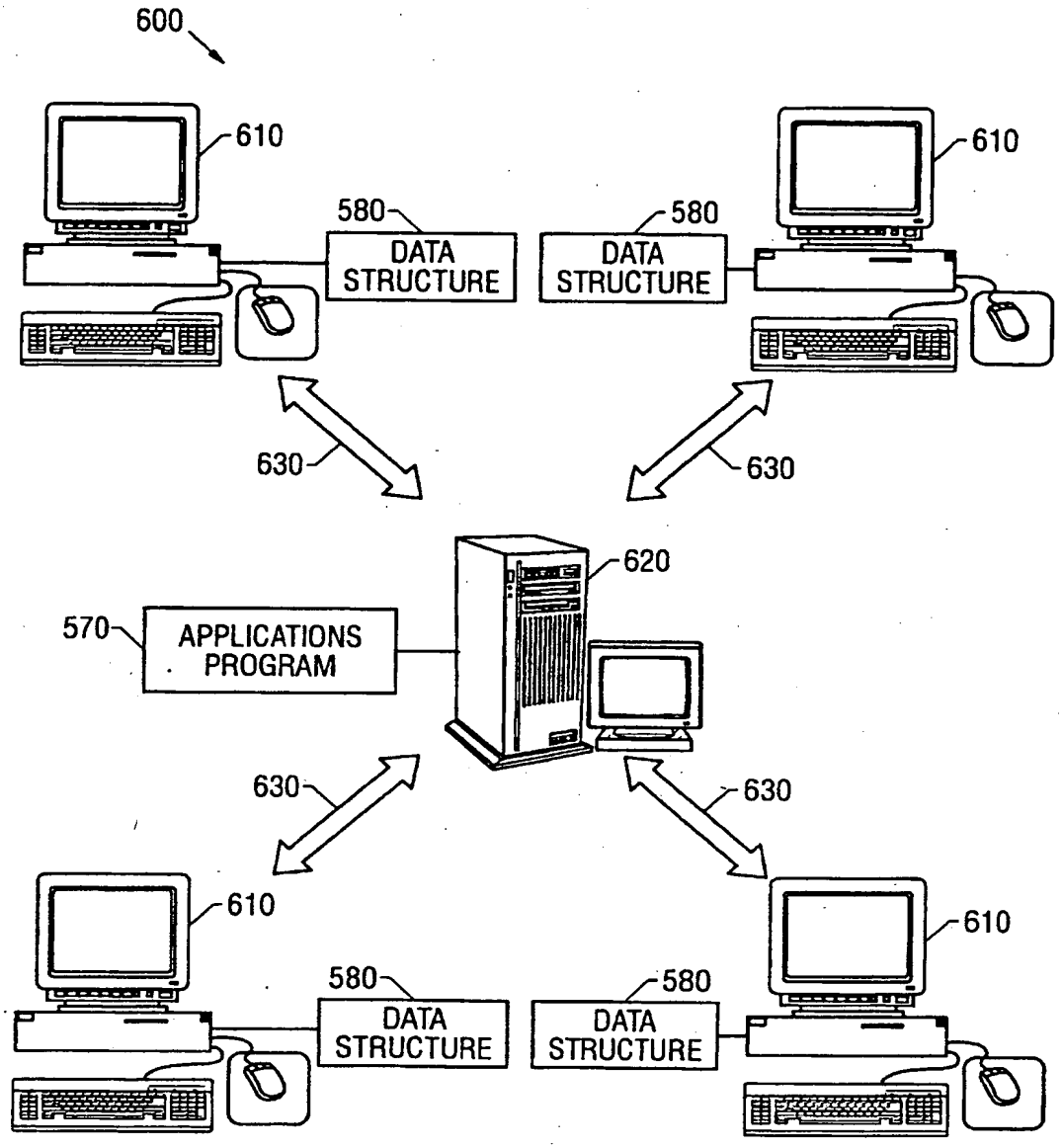


FIG. 7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580
DATA STRUCTURE
610
580
DATA STRUCTURE

FIG. 8

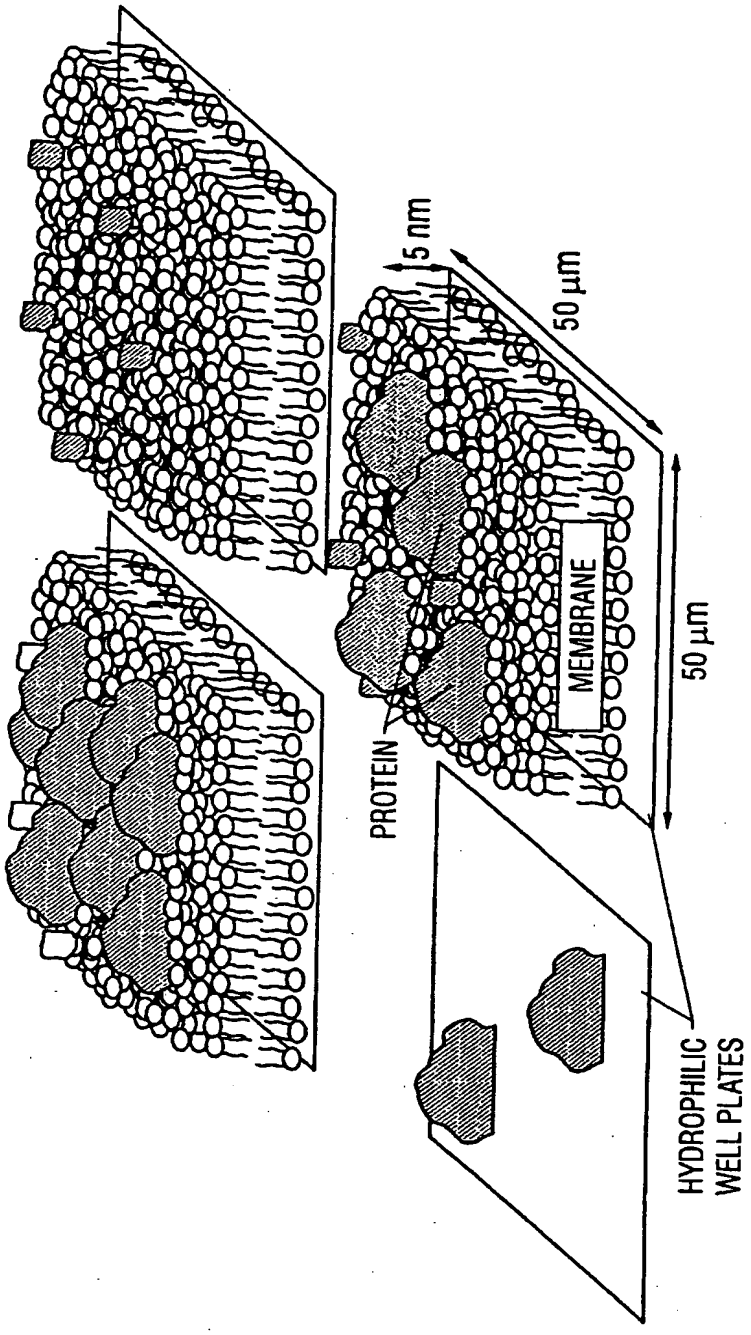

FIG. 9

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/25627

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/543 B01J19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N B01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, COMPENDEX, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 23948 A (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 4 June 1998 (1998-06-04) cited in the application the whole document	1,3-23, 27-30, 34-48, 56,57
A	WO 98 53092 A (MOTOROLA INC.) 26 November 1998 (1998-11-26) abstract page 11, line 18 -page 12, line 22 figures 7,8	2,49

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 January 2001	06/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Stevnsborg, N

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DAVID W. BRANDT: "MULTIPLEXED NANOLITER TRANSFERS FOR HIGH THROUGHPUT DRUG SCREENING USING THE BIOMEK 2000 AND THE HIGH DENSITY REPLICATING TOOL" JOURNAL OF BIOMOLECULAR SCREENING, vol. 2, no. 2, 1997, pages 111-116, XP000884553 NY, US ISSN: 1087-0571 figure 2	23-25
A	US 5 925 732 A (DAVID J. ECKER ET AL.) 20 July 1999 (1999-07-20) abstract; figure 6	
T	JENNIFER S. HOVIS & STEVEN G. BOXER: "Patterning barriers to lateral diffusion in supported lipid bilayer membranes by blotting and stamping" LANGMUIR, vol. 16, no. 3, 8 February 2000 (2000-02-08), pages 894-897, XP002158177 WASHINGTON, DC, US cited in the application figure 1	2,31-33
T	TINGLU YANG ET AL.: "Creating Addressable Aqueous Microcompartments above Solid Supported Phospholipid Bilayers Using Lithographically Patterned Poly(dimethylsiloxane) Molds" ANALYTICAL CHEMISTRY, vol. 72, no. 11, 2000, pages 2587-2589, XP002158178 AMERICAN CHEMICAL SOCIETY. COLUMBUS., US ISSN: 0003-2700 cited in the application the whole document	1-49,56, 57

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/25627

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		EP 0941474 A	15-09-1999
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		EP 0968303 A	05-01-2000
		GB 2330655 A	28-04-1999
US 5925732 A	20-07-1999	NONE	